Ecological and Genetic Indicators of Restoration Success

Alison L Ritchie
BSc (Hons)

THE UNIVERSITY OF WESTERN AUSTRALIA

This thesis is presented for the degree of Doctor of Philosophy of The University of Western Australia
School of Plant Biology
2015
SUMMARY

Restoration ecology is a rapidly growing science of global significance, assisting the recovery of ecosystems degraded, damaged or destroyed from human intervention and action. Increasingly, the focus is shifting beyond just restoring plant community structure to reinstating ecosystem functionality. A key process for sustainable plant communities is reproductive functionality, requiring robust pollinator services typically involving pollinators. Despite this, few restoration projects to date have explicitly assessed the restoration of pollinators. Pollinators are rarely specified in restoration targets as they are mobile and little is known about how they respond to habitat restoration, influence mating systems and reproductive success. The assumption is that once plant communities are established, pollinators and pollinator services will passively establish.

In this thesis, ecological studies of invertebrate and vertebrate richness, pollinator abundance and behaviour together with genetic analyses of pollen dispersal, mating and genetic structure using microsatellite DNA markers were conducted in several natural and restored Banksia (Proteaceae) woodland sites on the Swan Coastal Plain of Western Australia. Located within a global biodiversity hotspot, the Southwest Australian Floristic Region contains the highest proportion of vertebrate-pollinated plant species in the world with the largest number of nectar-feeding bird and mammal species. These Banksia woodlands have undergone severe habitat destruction with urban expansion and are situated on significant deposits for sand mining that have seen post-mining ecological restoration of variable standards. This provides a model system for testing and assessing the ecological genetic consequences of restoration in a fragmented landscape. The dominant species in these woodlands, Banksia attenuata and B. menziesii, depend solely on animal-mediated pollen flow to effectively reproduce, with nectarivorous birds (honeyeaters, Meliphagidae) and flying insects as key pollinators. By focussing on these species, this thesis assesses the restoration of reproductive functionality in restored plant communities by testing: (1) the effect of restoration on the diversity, abundance and behaviour of bird and insect pollinator communities, (2) the impact of pollinator behaviour on realised pollen dispersal, genetic landscape connectivity and reproductive output in B. menziesii, (3) the successful restoration and maintenance of genetic diversity and spatial genetic structure within restored and natural B. menziesii populations, and (4) the effects of high and low diversity restoration
approaches for genetic integration, connectivity and mating systems of *B. attenuata*. Studies were conducted within an array of natural bushland sites of differing levels of degradation and two restored sites, one with high plant species diversity and richness, and structural vegetation complexity reflective of pre-disturbance levels (high complexity), the second with low plant species diversity and richness (many non-native to the site), and vegetation structure of much lower complexity than found within pre-disturbance *Banksia* woodlands (low complexity).

Differences in initial plant species diversity of restored communities resulted in significant differences in pollinator species diversity and behaviour. However, this was not translated to an overall decreased reproductive output, due largely to the generalist pollinator requirements of these banksias. From a genetic perspective, the study species display resilience, as they are long-lived, completely outcrossing, show weak spatial genetic structure, and have generalist, highly mobile pollinators. The immediate outcomes from altered pollinator services in these sites are not detrimental to key population genetic processes. As such, these restored banksias are genetically integrated in the landscape through extensive gene flow with adjacent natural sites. A broader outcome of this genetic connectivity is the rapid immigration of non-local genes into neighbouring natural populations, the conservation consequences of which require further assessment. With depleting natural sources of seed for restoration, this study has implications for management decisions in anticipated future seed farming.
TABLE OF CONTENTS

SUMMARY .............................................................................................................................. 1
ACKNOWLEDGEMENTS .......................................................................................................... VII
DECLARATION OF CANDIDURE ........................................................................................... VIII
LIST OF FIGURES ................................................................................................................... IX
LIST OF TABLES ................................................................................................................... XVII
PREFACE ............................................................................................................................ XIX

CHAPTER 1: GENERAL INTRODUCTION ........................................................................ 1
Ecological restoration ........................................................................................................ 1
Evaluating the success of ecological restoration ............................................................... 1
Restoration of functioning ecosystems ............................................................................. 3
Role of pollinators in restoration ....................................................................................... 5
Genetic issues in restoration ............................................................................................. 6
Mating systems of restored populations ........................................................................... 7
Pollen dispersal and paternity assignment ........................................................................ 8
Spatial genetic structure of restored populations .............................................................. 9
This study ............................................................................................................................ 9

CHAPTER 2: BANKSIA WOODLAND RESTORATION: STUDY SITES, SPECIES
AND LANDSCAPE CONTEXT .............................................................................................. 13
Destruction and restoration of Southwest Western Australian biodiversity ..................... 13
Keystone species .............................................................................................................. 17
Banksia attenuata and Banksia menziesii ........................................................................... 18
Biology and ecology ......................................................................................................... 18
Study sites ......................................................................................................................... 20
Continuous Banksia woodland ........................................................................................... 23
Fragmented Banksia woodland .......................................................................................... 25
Natural Banksia woodland sites adjacent to restored sites ................................................. 29
Restored Banksia woodland ............................................................................................. 31
Field Sampling Techniques ............................................................................................... 34

CHAPTER 3: PLANT DIVERSITY INFLUENCES POLLINATOR ASSEMBLAGES
AND FORAGING BEHAVIOR IN RESTORED AND NATURAL BANKSIA
WOODLANDS. .................................................................................................................... 41
ABSTRACT ............................................................................................................................ 41
INTRODUCTION .................................................................................................................... 42
CHAPTER 5: AN EVALUATION OF GENETIC DIVERSITY AND SPATIAL GENETIC STRUCTURE IN RESTORED POPULATIONS OF BANXSIA MENZIESII

ABSTRACT .......................................................................................................................... 111
INTRODUCTION ................................................................................................................... 112
METHODS .............................................................................................................................. 115
Study species and populations .......................................................................................... 115
Characterization of microsatellite markers and genotyping ............................................ 117
DATA ANALYSIS ............................................................................................................... 117
Genetic diversity within sites and site differentiation....................................................... 117
Fine-scale spatial genetic structure .................................................................................. 118
RESULTS .............................................................................................................................. 119
Genetic diversity within sites ............................................................................................ 119
Site differentiation............................................................................................................. 123
Fine-scale spatial genetic diversity within populations ................................................... 124
DISCUSSION ........................................................................................................................... 127

CHAPTER 6: AN EVALUATION OF GENETIC DIVERSITY AND REPRODUCTIVE OUTPUT IN RESTORED POPULATION OF BANXSIA ATTENUATA ................. 133

ABSTRACT .......................................................................................................................... 133
INTRODUCTION ................................................................................................................... 134
METHODS .............................................................................................................................. 137
Study species ................................................................................................................... 137
Study sites ....................................................................................................................... 138
Site species diversity ........................................................................................................ 139
Flowering and fruiting: Assessment of reproductive output ............................................ 139
Genetic sampling .............................................................................................................. 139
Microsatellite genotyping ................................................................................................. 141
DATA ANALYSIS ............................................................................................................... 142
Site species diversity ........................................................................................................ 142
Genetic analysis ................................................................................................................ 142
Paternity assignment......................................................................................................... 143
Pollen pool structure and connectivity ............................................................................ 144
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spatial genetic structure</td>
<td>145</td>
</tr>
<tr>
<td>RESULTS</td>
<td>146</td>
</tr>
<tr>
<td>Site species diversity</td>
<td>146</td>
</tr>
<tr>
<td>Reproductive output</td>
<td>146</td>
</tr>
<tr>
<td>Genetic diversity</td>
<td>150</td>
</tr>
<tr>
<td>Population differentiation</td>
<td>153</td>
</tr>
<tr>
<td>Paternity assignment and realised pollen dispersal</td>
<td>154</td>
</tr>
<tr>
<td>Pollen pool differentiation</td>
<td>157</td>
</tr>
<tr>
<td>Spatial genetic structure</td>
<td>160</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>161</td>
</tr>
<tr>
<td>CHAPTER 7: GENERAL DISCUSSION</td>
<td>169</td>
</tr>
<tr>
<td>Targeting non-target species in restoration</td>
<td>172</td>
</tr>
<tr>
<td>New approaches to seed sourcing</td>
<td>176</td>
</tr>
<tr>
<td>Knowledge gaps and future research requirements</td>
<td>180</td>
</tr>
<tr>
<td>Concluding remarks</td>
<td>185</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>187</td>
</tr>
<tr>
<td>APPENDIX 1</td>
<td>220</td>
</tr>
<tr>
<td>APPENDIX 2</td>
<td>223</td>
</tr>
<tr>
<td>APPENDIX 3</td>
<td>225</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

I am very grateful to many people who have supported me and contributed their time to make this project possible. I wish to thank my supervisors, Siegy Krauss, Elizabeth Sinclair, Paul Nevill and Kingsley Dixon, for imparting their scientific knowledge, encouraging me through motivating conversations and providing constructive comments and guidance throughout my PhD. I am grateful to my main supervisor, Siegy, for providing me the opportunity to expand and develop my ideas. Thanks go to Michalie Foley for getting me started in research, mentoring me within my undergrad at Kings Park, which developed into my honours and then PhD.

My project would not have been possible without all my field volunteers, with special thanks to Kelly Rayner, Melanie Hunt, Kim Nguyen and Sarah Fairbass. Words cannot express how grateful I am to my mother for the many days every year getting up before sunrise to head out into the bush for 35+ degree-days, in order to help collect my data.

A good support system is vital to surviving a PhD, and heartfelt thanks must go to my family; Dad, Donald and Hamish, and friends; Emma Dalziell, Todd Erickson, Jessica Stingemore, Bryn Funnekotter, Wolfgang Lewandrowski, Anna Williams, Wei-han Lim, Myles Menz, Donna Bradbury and Jessie Roxby. They have all helped me tremendously in the field and in the office. They have been there for the highs and lows, laughing with me in the highs, and at me in my clumsy lows (e.g. bee stings, bogged cars and mechanical failures) where the phrase “it’s Ali” is synonymous. I would like to thank Carole Elliott, arriving near the end of my PhD she has been my ‘Mary Poppins’, providing an ear, a shoulder, a slice of cake and sound advice when needed. To all the staff in Kings Park Science, particular thanks go to Janet Anthony for guidance and advice on all things genetic, and Annette Johnson for all things organizational.

This research was supported by the School of Plant Biology, The University of Western Australia Postgraduate Travel Award, Botanic Gardens and Parks Authority, and Hollsworth Wildlife Research Endowment. Thank you to Rocla Quarry Products and City of Wanneroo for permission to conduct research on their land.
DECLARATION

DECLARATION FOR THESSES CONTAINING PUBLISHED WORK AND/OR WORK PREPARED FOR PUBLICATION

1. This thesis **does not contain** work that I have published, nor work under review for publication.

Student Signature

__________________________________________________________________________________________
LIST OF FIGURES

Figure 2.1 Changes in vegetation cover on the Swan Coastal Plain (SCP) between 1990-1992 and 1990-2013. (Modified vegetation change maps accessed from Land Monitor, Western Australian Land Information Authority – Landgate, accessed Feb 2014: http://landmonitor-beta.langate.wa.gov.au). .......................................................... 15

Figure 2.2 Changes in vegetation cover in northern Perth between 1992 and 2013. Images contain study areas (Alexander Park, Highview Park, Marangaroo Conservation Area and Paloma Park) (modified vegetation change maps accessed from Land Monitor Western Australia, Land Information Authority– Landgate, accessed Feb 2014: http://landmonitor-beta.langate.wa.gov.au). .......................................................... 16

Figure 2.3 Banksia attenuata (top) and Banksia menziesii (bottom) trees in flower. .... 19

Figure 2.4 Banksia attenuata (above) and Banksia menziesii (left) of inflorescences to infructescence (cone) succession stages. Photographs taken by author......................... 21

Figure 2.5 Ten study site locations on the Swan Coastal Plain, Western Australia; A: NN, Neaves North, NS, Neaves South; B: GN, Gnangara natural, GR, Gnangara restored; C: AP, Alexander Park, HP, Highview Park, PP, Paloma Park, MC, Marangaroo Conservation Area; D: JN, Jandakot natural, JR, Jandakot restored......... 22

Figure 2.6 Continuous Banksia woodland showing sparse arrangement of overstorey trees. Photograph taken in the middle of the site, by author. ................................. 23

Figure 2.7 Alexander Park Banksia woodland showing rich species diversity. Photograph taken in the middle of the site, by author. .......................................................... 25

Figure 2.8 Highview Park Banksia woodland showing tree deaths and disturbance. Photograph taken in the middle of the site, by author. ................................. 26

Figure 2.9 Paloma Park Banksia woodland showing disturbance. Photograph taken in the middle of the site, by author. .......................................................... 27

Figure 2.10 Marangaroo Conservation Area Banksia woodland showing tracks and disturbance. Photograph taken in the middle of the site, by author. ................................. 28
Figure 2.11 Gnangara natural, adjacent Banksia woodland to Gnangara restored site. Photograph taken in the middle of the site, by author.................................................................29

Figure 2.12 Jandakot natural, adjacent Banksia woodland to Jandakot restored site, including Malaise Trap. Photograph taken in the middle of the site, by author. .................31

Figure 2.13 A sand extraction mine site with Banksia woodland in the background. Photograph taken by author........................................................................................................32

Figure 2.14 Gnangara restored Banksia woodland. Photograph taken in the middle of the 1996 restored site, by author. ..........................................................................................33

Figure 2.15 Jandakot restored Banksia woodland showing lack of understorey species and leftover reticulation piping. Photograph taken in the middle of the 1995 restored site, by author. ........................................................................................................34

Figure 2.16 Growth stages from (Hnatiuk RJ et al., 2009). .............................................35

Figure 2.17 Principal components analysis (PCA) plot of measured environmental variables (Table 2.3) using Euclidean distances, characterizing study sites of Banksia menziesii along the Swan Coastal Plain. Created in PRIMER v 6 (Clarke, 1993) AP, Alexander Park; HP, Highview Park; MC, Marangaroo Conservation Area; PP, Paloma Park; NN, Neaves North; NS, Neaves South; GN, Gnangara natural; GR; Gnangara... 36

Figure 3.1 Diagram of a Malaise Trap tent structure and dimensions. Inset (left) is a photograph of the killing agent, Vapona (yellow cube) and insects captures within the bottle (photographed by author). .....................................................................................50

Figure 3.2 Natural (above) and restored (below) photographs of Malaise Traps during Banksia attenuata (left) and Banksia menziesii (right) flowering (photographed by author). ........................................................................................................................51

Figure 3.3 Non-metric multidimensional scaling (NMDS) plots (resemblance by Bray-Curtis similarity) showing clustering of presence and abundance of species in terms of flowering Banksia species, B. attenuata (summer) and B. menziesii (winter). A, displays invertebrates sampled from Malaise Traps and B, pollinating species observed from point counts. AP, Alexander Park; HP, Highview Park; PP, Paloma Park; MC,
Figure 3.4 Non-metric multidimensional scaling (NMDS) plots (resemblance by Bray-Curtis similarity) showing clustering of invertebrate functional types by site type. Species sampled from Malaise Traps and combined for flowering banksias. Overlaid clusters indicate similarity at levels of 60% (black line) and 75% (blue line). AP, Alexander Park; HP, Highview Park; PP, Paloma Park; MC, Marangaroo Conservation Reserve; NN, Neaves North; NS, Neaves South; GN, Gnangara natural; JN, Jandakot natural; GR, Gnangara restored and JR, Jandakot restored.

Figure 3.5 Nectarivores birds observed in the Banksia woodlands of the Swan Coastal Plain, A: *Anthochaera carunculata* (Red Wattlebird), B: *Anthochaera lunulata* (Western Wattlebird), C: *Trichoglossus haematodus* (Rainbow Lorikeet), D: *Phylidonyris nigra* (White-cheeked Honeyeater), E: *Phylidonyris novaehollandiae* (New Holland Honeyeater), F: *Lichenostomus virescens* (Singing Honeyeater), G: *Acanthorhynchus superciliosus* (Western Spinebill), H: *Lichmera indistinct* (Brown Honeyeater) and *Zosterops lateralis* (Silvereye or Wax-eye).

Figure 3.6 Non-metric multidimensional scaling (NMDS) plots (resemblance by Bray-Curtis similarity), showing clustering of presence and abundance of pollinators (bird and invertebrate) from observed point counts for both flowering species *Banksia attenuata* (summer) and *B. menziesii* (winter), produced from NMDS using Bray-Curtis dissimilarity measure with overlaid clusters at a similarity level of 60% (black line) and 75% (blue line). AP, Alexander Park; HP, Highview Park; PP, Paloma Park; MC, Marangaroo Conservation Area; NN, Neaves North; NS, Neaves South; GN, Gnangara natural; JN, Jandakot natural; GR, Gnangara restored and JR, Jandakot restored.

Figure 3.7 Banksia woodland sites: Non-metric multidimensional scaling (NMDS) of pollinator abundance between sites with superimposed ‘bubble’ plots indicating observed visitation of; Red Wattlebirds, Western Wattlebirds, New Holland Honeyeaters, Brown Honeyeaters, Western Spinebills, White-cheeked Honeyeaters, European Honeybees, and Native bees. Circle size corresponds to the number of foraging counts surveyed within each site and colour corresponds to site type.

Figure 3.8 Differences in pollinator visitation among site type and flowering season: summer flowering *Banksia attenuata* (top) and autumn/winter flowering *Banksia*
**menziesii** (bottom). Mean (± SE) numbers of visits were recorded per 10-minute survey every hour for birds, native insects, and invasive introduced European Honeybees. Visits were recorded for 10 minutes every hour within 30 minutes after sunrise with four surveys each flowering season, at each site, over 3 years (2010-2013).  

Figure 3.9 The proportions of intra-tree (I), near neighbor (N), distant (D), to *Adenantheros* (shrub species) (A) and those that flew out of the site (F) movements by honeyeaters between foraging bouts on *Banksia attenuata* and *B. menziesii* inflorescences within fragmented, continuous and restored sites.  

Figure 3.10 Non-metric multidimensional scaling (NMDS) ordination of pollinator movements during and after foraging bouts on *Banksia attenuata* and *B. menziesii* inflorescences within fragmented, continuous and restored sites AP, Alexander Park; HP, Highview Park; PP, Paloma Park; MC, Marangaroo Conservation Reserve; NN, Neaves North; NS, Neaves South; GN, Gnangara Natural; JN, Jandakot Natural; GR, Gnangara Restored and JR, Jandakot Restored.  

Figure 3.11 Aggressive chases by birds recorded in all study sites and *Banksia* species flowering seasons. Numbers within circles indicate intraspecific chases and arrows indicate direction of chases. Circle size indicates proportional body weight (g), sourced from Ford (1979), Newland and Wooller (1985) and McFarland (1986). Bird names in **bold** text are nectarivores.  

Figure 4.1 Mean inflorescence (a), follicle production (b) and follicle to inflorescence ratio (c) per plant in *Banksia menziesii* sites. Data were collected 2010, 2011 and 2012. Annual rainfall for each site (a) obtained from BOM (www.bom.gov.au). AP, Alexander Park; HP, Highview Park; PP, Paloma Park; MC, Marangaroo Conservation Reserve; NN, Neaves North; NS, Neaves South; GN, Gnangara Natural; JN, Jandakot Natural; GR, Gnangara Restored and JR, Jandakot Restored. Data were not collected at JN and JR in 2010. Standard error bars are shown.  

Figure 4.2 Positive association between (a, b, c) inflorescence number and follicle production for each of three years, (d) pollinator visitation and inflorescence production, and (e) pollinator visitation and follicle production in *Banksia menziesii*. Five sites were measured in 2010, and 7 sites in 2011 and 2012. Linear regression lines and the results of regression analyses are shown. n = number of plants measured.
Figure 4.3 Conditional genetic covariance among pollen pools for A, continuous natural site, Neaves North; B, fragmented sites within an urban landscape, Alexander Park and Highview Park; C, Gnangara adjacent natural and restored; D, Jandakot adjacent natural and restored, depicted as Pollination Graphs overlayed on a satellite image of sites and in 3-dimensional space. Nodes represent the population of pollen haplotypes sampled by each maternal individual. Edges represent significant statistical covariance among connected pollen pools. ................................................................. 101

Figure 4.4 Positive associations between (a) inflorescence number and within family genetic variation (node size) and (b) follicle production and within family genetic variation (node size) in Banksia menziesii. Reproductive output was measured for each maternal tree in 2010 within each site (n = 70). Linear regression lines and the results of regression analyses are shown................................................................. 104

Figure 5.1 Aerial photographs of continuous, fragmented and restored populations of Banksia menziesii assessed for genetic diversity and mating pattern parameters. Each circle indicated the position of a sampled tree, White circles indicate maternal tree in which seed was collected................................................................. 116

Figure 5.2 Allelic diversity and heterozygosity across sites of Banksia menziesii adults and offspring. Na, average number of alleles per locus; Ne, effective number of alleles; I, Shannon’s Information Index; He, average expected heterozygosity. AP, Alexander Park; HP, Highview Park; NN, Neaves north; GN, Gnangara natural; JN, Jandakot natural; Gnangara restored and JR, Jandakot restored. ................................................ 122

Figure 5.3 Spatial autocorrelation analysis correlograms for populations of Banksia menziesii, showing the genetic correlation coefficient (r) for increasing distance class sizes with 95% confidence intervals about r as determined by bootstrapping (in red). (A, Alexander Park, B, Highview Park, C, Gnangara natural, D, Gnangara restored, E, Jandakot natural, F, Jandakot restored and G, Neaves north). ................................. 126

Figure 6.1 Location of Jandakot study site, southwest of Perth, Western Australia. Circles indicate Banksia attenuata trees; open circles indicate the location of sampled natural trees (green) and restored trees (orange); closed circles indicate sampled maternal trees in the natural (green) and restored (orange) site. ................................. 140
Figure 6.2 Location of Gnangara comparison site (Ritchie & Krauss, 2012) northwest of Perth, Western Australia. Circles indicate Banksia attenuata trees; open circles indicate the location of sampled natural trees (green) and restored trees (orange); closed circles indicate sampled maternal trees in the natural (green) and restored (orange) site.

Figure 6.3 Photographs illustrating structural complexity at each site; A, Jandakot restored; B, Jandakot natural; C, Gnangara restored; D, Gnangara natural. Taken by author.

Figure 6.4 Number of plant species present in reference and restored Banksia woodland sites.

Figure 6.5 Reproductive output per plant in Banksia attenuata populations (a) mean inflorescence, (b) follicle production and (c) follicle to inflorescence ratio. Annual rainfall for each site (a) obtained from BOM (www.bom.gov.au). Data were not collected at JN and JR in 2010. Standard error bars are shown.

Figure 6.6 Relationship between inflorescences and follicle production in Banksia attenuata sites were measured in (a) 2011 and (b) 2012. Linear regression lines and the results of regression analyses are shown. n = number of plants measured.

Figure 6.7 Allelic variation for natural and restored adult and offspring populations of Banksia attenuata at Jandakot. Na, Average number of alleles per locus; Ne, effective number of alleles; I, Shannon’s Information Index; Pr, number of private alleles and He, average expected heterozygosity adjusted for sample size.

Figure 6.8 Map of Banksia attenuata trees in the natural (top; squares) and restored (bottom; circles) sites at Jandakot, indicating the 10 mother trees from which seed was sampled. Filled squares and circles indicate trees that were genotyped. Each pie contains 10 genotyped seed collected in 2011, labeled with the mothers ID. Each segment indicates the source location of the pollen donor tree: natural (green), restored (orange), or unassigned (white). Joined segments indicate a shared pollen donor.

Figure 6.9 Distance class distributions of pollen flow inferred from parentage analysis of Banksia attenuata seeds sourced from Jandakot natural and restored sites. Paternity analysis based on relative LOD scores to potential fathers of 43 offspring sourced from the natural site and 41 offspring from the Jandakot restored site. Sires from the natural site are in green and sires from restored site are in orange bar graphs.
Figure 6.10 Conditional genetic covariance among pollen pools for Jandakot natural and restored sites; A, Pollination Graphs overlaid on a satellite image of the site and B, depicted in 3-dimensional space. Nodes represent the population of pollen haplotypes sampled by each maternal individual. Edges represent significant statistical covariance among connected pollen pools. 

Figure 6.11 Spatial autocorrelation analysis correlograms for Banksia attenuata at Jandakot Natural (green) and Jandakot Restored (orange) showing the genetic correlation coefficient (r) for increasing distance class sizes, with 95% confidence intervals about r as determined by bootstrapping.

Figure 7.1 Forested sites in good and excellent condition supported diverse bird communities. In general, the downward shift from medium to poor ecological condition as defined by bird communities coincided with a shift in land cover composition from forested to non-forested in the Appalachians, Eastern United States of America. Taken from (O'Connell et al., 2000).

Figure 7.2 Spatial arrangement of natural habitat patches (shaded), and insertion of small restored habitat patch (black) in two different scenarios of (a) and (b). Different locations can result in very different effects on functional connectivity and the corresponding habitat network. Adapted from Villard and Metzger (2014).

Figure 7.3 Reproductive functionality - the next link in the chain for successful ecological restoration. Adapted from Merritt and Dixon (2011).

Figure 7.4 Native Banksia woodland is being rapidly clearing for housing on the Swan Coastal Plain. Photographs taken by author.
LIST OF TABLES

Table 2.1 Proportion of Western Australian terrestrial fauna within Banksia woodlands (Knowles, 2011). Sourced from Banksia Woodland Symposium Proceedings 2011 .... 17

Table 2.2 Indicators of growth stage .................................................................................................................. 35

Table 2.3 Site characteristics and measurements of Banksia menziesii tree plant density, average diameter at breast height (DBH), height, health, and growth stages within each study area. Confidence intervals are shown in parentheses. ........................................ 37

Table 2.4 Sites used for each study and related chapter................................................................................. 38

Table 3.1 Site characteristics and mean inflorescence count per tree for 10 trees in each site (95% confidence intervals) for Banksia attenuata and B. menziesii between 2010 – 2013. No results were recorded for B. attenuata flowering in JN and JR for summer 2010/2011............................................................ 49

Table 3.2 Species richness for taxa of all insects captured by Malaise Traps and diversity indices for each site. Details of insect floral visitors are listed within the appendices (see also Appendix 1 for more detailed classifications). ......................... 56

Table 4.1 Reproductive measures of Banksia menziesii in continuous, fragmented, adjacent and restored sites. Average number of follicles produced per tree, follicles setting viable seed and the percentage of viable seeds averaged per maternal over all years (and their 95% confidence intervals shown in parentheses).......................... 95

Table 4.2 Mating system and pollen gene pool parameters estimated for seven sites of Banksia menziesii (95% confidence interval in parentheses) ..................................................... 97

Table 4.3 Simulation of paternity assignment (father given known mother) at each site under strict (95%) and relaxed (80%) confidence levels. Critical LOD (natural logarithm of the likelihood-odd ratio), number of observed assignments, assignment rate, maximum PDD (pollen dispersal distance) and Aep, estimated effective pollen area radius, centred on each maternal calculated from TwoGener results................................. 100

Table 5.1 Genetic diversity measures (95% confidence intervals in parentheses) in sites of Banksia menziesii .................................................................................................................. 120
Table 5.2 Analysis of molecular variance (AMOVA) for adult and offspring cohorts of *Banksia menziesii*. Variance was calculated between one restored site and one natural site in Gnangara and in Jandakot, and between natural sites, using 11 polymorphic microsatellite markers. .................................................................................................. 124

Table 5.3 Spatial genetic structure measures; $Sp$ statistic and Neighbourhood size ($Nb$) for sites of *Banksia menziesii* .................................................................................................................. 125

Table 6.1 Genetic diversity parameters and fixation index (95% confidence intervals in parentheses) for adult and offspring populations from both natural and restored populations of *Banksia attenuata* in Jandakot and Gnangara (Ritchie & Krauss, 2012). ....................................................................................................................................... 151

Table 6.2 Analysis of molecular variance (AMOVA) for adult and offspring populations of *Banksia attenuata*. Variance was calculated between one restored population and one natural population in Jandakot, using 7 polymorphic microsatellite markers. Statistics include sums of squared deviations (SSD), variance component estimates, percentage of total variance (% total) contributed by each component, and the probability ($P$) of obtaining a more extreme component estimate by chance alone................................. 153

Table 6.3 Mating system parameters (and their 95% confidence intervals shown in parentheses) for natural and restored sites of *Banksia attenuata*, estimated using open-pollinated offspring from 10 trees per site in Jandakot and 5 trees per site in Gnangara (data obtained from Ritchie & Krauss (2012))................................................................. 158
PREFACE

This thesis consists of a series of interrelated manuscripts, with the exception of Chapter 1 (introduction), Chapter 2 (Site descriptions) and Chapter 7 (Conclusion). The core data chapters of this thesis are intended to be stand-alone pieces of research for publication in scientific journals, therefore, some repetition between the chapters was unavoidable.
Chapter 1: General Introduction

Ecological restoration

Globally, the major threatening processes to natural ecosystems are anthropogenic through changes in land use, degradation and destruction (Dobson et al., 1997). A key strategy to reduce these damaging processes is to implement ecological restoration programs (Hobbs & Cramer, 2008; García-Robledo, 2010). Ecological restoration is the process of assisting the recovery of an ecosystem that has been degraded, damaged or destroyed, performed by practitioners (SER, 2004). In a recent declaration on the Conservation of Biological Diversity, the global community committed to a new target to restore 15% of degraded ecosystems worldwide by 2020 (Jørgensen, 2013). To meet these targets, a rapid growth of the restoration industry, and recognition of its importance, complexity and challenges, is required (Roberts et al., 2009; Aronson & Alexander, 2013). Restoration ecology is the science underpinning the practice of ecological restoration, and uses restoration to advance ecological theory (Falk et al., 2006).

The definition of ecological restoration from the Society for Ecological Restoration International (SER) is “the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed” (SER, 2004). Restoration objectives vary, but best practice often aims to establish biological communities that are sustainable and representative of the composition, diversity and functionality of the pre-disturbance habitat (Holl et al., 2003; Hufford & Mazer, 2003; Dolan et al., 2008). Restoration practices can range from adding individuals to rehabilitate impacted populations (species level), to establishing new communities of species (community level), to addressing remedial issues such as salinity or land instability, with the goal of replacement or the development of an acceptable new ecosystem on the managed site (Falk et al., 2006; Hobbs & Cramer, 2008).

Evaluating the success of ecological restoration

Whilst ecological restoration is being undertaken at large scales, there is still uncertainty in how effective the ecological restoration programs have been (Suding, 2011). This can
be attributed to the discipline’s age (less than 30 years) (Young et al., 2005) in comparison to the timescale of ecological processes to develop (possibly hundreds of years) (Wortley et al., 2013), as well as unrealistic or poorly defined goals, inadequate restoration plans, lack of explicitly quantified evaluation criteria for monitoring of restoration, and a general lack of ecological understanding (Choi, 2004; Miller & Hobbs, 2007; McDonald & Williams, 2009; Suding, 2011; Parkes et al., 2012). Determining a ‘desirable’ trajectory for restored sites is often challenging, with the unpredictability of ecological communities in a changing environment, and especially within a disturbed system (Hobbs & Norton, 1996). The Society for Ecological Restoration SER (2004) describes that “in restoration, the trajectory begins with the unrestored ecosystem and progresses towards the desired state of recovery expressed in the goals of a restoration project and embodied in the reference ecosystem”. In most cases, the ultimate goal is to return an ecosystem to a close approximation of its condition prior to disturbance. In order to do so, we must determine how the reference ecosystem functions. However, our understanding of natural processes, despite our large advancement in recent centuries, is still limited (Hobbs & Norton, 1996; Dobson et al., 1997; Suding, 2011) and the timescale of restoration assessments through monitoring are largely too limited to discern if these goals have been reached (Dobson et al., 1997; Suding, 2011).

Evaluating restoration success is not straightforward, with many authors developing and applying different approaches (Pielou, 1986; Pywell et al., 2003; Ruiz-Jaén & Aide, 2005b; Ruiz-Jaén & Aide, 2005a; Tischew et al., 2010). Restoration frameworks provide help to define and assess restoration goals (Hobbs & Norton, 1996; McIntyre & Hobbs, 1999; Choi, 2004; Suding & Hobbs, 2009), however for species rich ecosystems, we need more empirical data to feed into these models to gain knowledge on ecosystem functioning. Assessments of restoration success have historically focused on structural properties of restored ecosystems such as vegetation structure, species diversity and abundance (Ruiz-Jaén & Aide, 2005b; Ruiz-Jaén & Aide, 2005a). This is understandable, since most restoration programs are cost and time constrained, requiring rapid, sensitive and economically feasible assessment tools, which can be carried out by restoration practitioners (Lomov et al., 2006).

In general, it is assumed that the colonization of fauna follows the establishment of restored plant communities (Handel, 1997; Palmer et al., 1997; Majer, 2009; Williams,
However, a myriad of processes such as anthropogenic habitat fragmentation, spatial isolation from relatively undisturbed plant communities, and/or habitat heterogeneity effect the reestablishment of pollination services (Winfree et al., 2011). The realization that the success of restoration was intimately linked to vertebrate and invertebrate fauna became apparent with increased environmental research in the 1990s (Majer, 2009). Research in this area has since increased rapidly, investigating the roles that fauna play in restoration, such as the importance of decomposers, the impact of herbivores, the role of pollinators, seed dispersers, and predators, and the potential value of groups of animals as bio-indicators (O’Connell et al., 2000; Dale & Beyeler, 2001; Lomov et al., 2006; Mayer et al., 2012). There has been a move from the ad-hoc gardening approach of the past to a ‘futuristic’ approach with a realistic set of goals that are not static (Box 1.1, Choi, 2004), and more recently look beyond ecology, including moral, social, economic and cultural aspects within restoration targets (Wortley et al., 2013). Ecological restoration is not just a matter of planting trees, it is the restoration, recovery and improvement of functioning ecosystems to support multiple ecosystem services (Herrick et al., 2006).

**Restoration of functioning ecosystems**

There is a growing acknowledgement that sustainable restoration requires the restoration of functioning ecosystems (Wortley et al., 2013). The move to focus on re-instating ecological functions or processes is to restore the dynamic attributes of ecosystems, which include the interactions within and among organisms in their environment (SER, 2004). Ecosystem functions are often defined as the attributes that affect metabolism, sequestration and transformation of energy and nutrients, and therefore ecosystem processes are limited to the attributes based in the self-maintenance in an ecosystem, such as differentiation of habitat for specialized species, pollination and seed dispersal (SER, 2004).

Ecosystem functionality is generally conceived at larger spatial scales, such as the long-term retention of nutrients and moisture and overall ecosystem sustainability (SER, 2004). Restoring functionality is often considered a loftier goal that practitioners struggle to accomplish due to the lack of proven techniques for its realization (Kettenring et al., 2014). Few studies have evaluated restoration success from a functional perspective (Ruiz-Jaén & Aide, 2005a; Ruiz-Jaén & Aide, 2005b; Lomov et
al., 2010; Ritchie & Krauss, 2012; Baer et al., 2014). However, the shift to address ecological restoration at a larger community-functioning level is apparent. Attributes of functionality, which are vital for successful and sustainable ecological restoration include the creation of genetically diverse ecosystems that provide for the delivery of pollinator services.

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td><strong>Attribute</strong></td>
</tr>
<tr>
<td>1</td>
<td>The restored ecosystem contains a characteristic assemblage of the species that occur in the reference ecosystem and that provide appropriate community structure.</td>
</tr>
<tr>
<td>2</td>
<td>The restored ecosystem consists of indigenous species to the greatest practicable extent. In restored cultural ecosystems, allowances can be made for exotic domesticated species and for noninvasive ruderal and segetal species that presumably co-evolved with them. Ruderals are plants that colonize disturbed sites, whereas segetals typically grow intermixed with crop species.</td>
</tr>
<tr>
<td>3</td>
<td>All functional groups necessary for the continued development and/or stability of the restored ecosystem are represented or, if they are not, the missing groups have the potential to colonize by natural means.</td>
</tr>
<tr>
<td>4</td>
<td>The physical environment of the restored ecosystem is capable of sustaining reproducing populations of the species necessary for its continued stability or development along the desired trajectory.</td>
</tr>
<tr>
<td>5</td>
<td>The restored ecosystem apparently functions normally for its ecological stage of development, and signs of dysfunction are absent.</td>
</tr>
<tr>
<td>6</td>
<td>The restored ecosystem is suitably integrated into a larger ecological matrix or landscape, with which it interacts through abiotic and biotic flows and exchanges.</td>
</tr>
<tr>
<td>7</td>
<td>Potential threats to the health and integrity of the restored ecosystem from the surrounding landscape have been eliminated or reduced as much as possible.</td>
</tr>
<tr>
<td>8</td>
<td>The restored ecosystem is sufficiently resilient to endure the normal periodic stress events in the local environment that serve to maintain the integrity of the ecosystem.</td>
</tr>
<tr>
<td>9</td>
<td>The restored ecosystem is self-sustaining to the same degree as its reference ecosystem, and has the potential to persist indefinitely under existing environmental conditions. Nevertheless, aspects of its biodiversity, structure, and functioning may change as part of normal ecosystem development, and may fluctuate in response to normal periodic stress and occasional disturbance events of greater consequence. As in any intact ecosystem, the species composition and other attributes of a restored ecosystem may evolve as environmental conditions change.</td>
</tr>
</tbody>
</table>
Several studies have focused on identifying biological indicators or flagship species that identify with a threatening process i.e. are sensitive to environmental stress or can be identified with the establishment of ecosystem processes (Dale & Beyeler, 2001; Lomov et al., 2006; Majer, 2009). These focal-species can then be a target for the management of threatening processes and vegetation-based restoration efforts (Lindenmayer et al., 2002a). Unless these indicators have been recognized, for example, pollinators, the assessment of the reestablishment of pollination services within restoration remains untargeted (Williams, 2011).

**Role of pollinators in restoration**

Pollination is a fundamental ecosystem process for the persistence of plant and animal populations (Bond, 1994; Kearns et al., 1998; Forup et al., 2008). Therefore, restoration of plant-pollinator interactions is essential for ecosystem recovery (García-Robledo, 2010). Approximately 300,000 plant species (87% of flowering plants) require animal-mediated pollination, and mutualistic relationships occur between plants and over 200,000 vertebrate and invertebrate pollinators worldwide (Kearns et al., 1998; Burkle & Alarcon, 2011; Ollerton et al., 2011; Winfree et al., 2011). This dependence on pollinators to maintain self-sustainability through reproduction and genetic viability needs to be more explicitly incorporated into restoration (Forup et al., 2008; Dixon, 2009; Menz et al., 2011). Despite its significance, the ecological restoration of plant-pollinator interactions has only recently begun to be investigated (Menz et al., 2011; Winfree et al., 2011). For example, only 265 papers of a total of 674 studies in existence of pollinator responses to anthropogenic land use have been published (Winfree et al., 2011).

The re-establishment of pollinators into restored plant communities usually requires movement from remnant natural sources that often suffer from impacts such as habitat fragmentation. Predicting and managing the effects of human-induced habitat disturbance is particularly challenging in organisms that rely on interactions with other species for services such as pollination and dispersal (England et al., 2001; Menz et al., 2011). There is increasing evidence that human activities and disturbance negatively influence and impact plant-pollinator interactions such as outcross pollination (Eckert et al., 2010). These pollinator interactions may not re-establish themselves in restored plant communities, as pollinators require additional provisions, such as nesting sites or
food resources (Forup et al., 2008). This leads to the question, if we re-vegetate, will the required pollinator services re-establish naturally (without active intervention)?

Relatively few plant-pollinator interactions are absolutely obligate and most are generalized (Waser et al., 1996). A key component of population functionality for outcrossing plant species is the delivery of robust pollinator services for the avoidance of inbreeding and the production of outbred offspring (Moeller, 2004), consequently achieving genetic self-sustainability and resilience through the avoidance of an erosion of genetic diversity. Populations with a reduced facility for outcrossing due to ineffective delivery of pollinator services, exhibit eroded genetic diversity, which can lead to decreased seed set, increased inbreeding, and decreased population fitness (Groom, 1998; Ashman et al., 2004; Wooller & Woller, 2004; Heliyanto et al., 2006). The presence of pollinators (such as nectarivorous birds (Honeyeaters, Meliphagidae)) does not necessarily mean that the ecological function of pollination they are normally associated with has been attained to the desired (pre-disturbance) state in these restored areas (Majer, 2009). It is thus imperative that field-based studies are conducted that links the abundance or diversity of pollinators to the degree to which they are carrying out the ecological function of pollination (Loreau et al., 2001). This can be evaluated through observations of foraging behaviour accompanied by genetic studies of pollen flow, and seed set, in restored and natural sites.

**Genetic issues in restoration**

The genetic issues impacting on the success of ecological restoration activities, such as, provenance of source material, evolutionary potential, local adaptation and inbreeding or outbreeding depression within founding plants and genetic swamping of remnant local populations with introduction of non-local plants (Hufford & Mazer, 2003; Williams et al., 2014) are being addressed and incorporated into restoration activities (Lesica & Allendorf, 1999; Rogers & Montalvo, 2004; Reusch et al., 2005; Young et al., 2005; Bischoff et al., 2010). Ecological genetic issues are fundamental to the success of a restoration project as they underpin how species function within ecosystems (Falk et al., 2006).

Founder effects are likely to occur if the plant material used to restore a site (seeds, seedlings, tissue culture) is from a limited number of source plants (Gustafson et al.,
Chapter 1: General Introduction

2002; Broadhurst et al., 2008a; Sinclair & Hobbs, 2009; Bischoff et al., 2010). The genetic diversity of these founders may represent only a small portion of the allelic diversity present within the source population, and as a result genetic bottlenecks could develop within the restored populations, especially if the population is isolated from other sources of gene flow and the population size is small (Montalvo et al., 1997; Lesica & Allendorf, 1999; Hufford & Mazer, 2003; Pierson et al., 2007; Kettle et al., 2008).

The long-term fitness of a restored population will be affected by the source of seed (Herath et al., 2009; Bischoff et al., 2010), and the degree to which they are adapted to the restoration environment (Lesica & Allendorf, 1999; Broadhurst et al., 2006; Kettle et al., 2008). Common practice for seed sourcing generally promotes the use of locally adapted seeds from multiple sources to preserve local genotypes and to maximise genetic diversity (Krauss et al., 2007; Breed et al., 2013), although this is dependent on the ultimate objectives of an individual restoration project (Lesica & Allendorf, 1999; Broadhurst et al., 2008b). The use of molecular markers provides a powerful approach for the delineation of the extent of the local provenance (Bussell et al., 2006; Krauss et al., 2013), as well as for the assessment of the degree of genetic integration of plants within a restored population and adjacent remnants. Genetic integration may be important for avoiding potentially detrimental impacts in future generations such as outbreeding depression (Hufford & Mazer, 2003; Hufford et al., 2012; Williams et al., 2014).

**Mating systems of restored populations**

Focus must ultimately be placed not only the genetic variation of the founding population, but also the genetic variation in the offspring of the founders. The levels of future genetic variation in a restored population will be affected by the genetic diversity of the founders and the patterns of mating between them (Krauss et al., 2002; Pierson et al., 2007; Fant et al., 2008). Genetic assessment of the offspring of founders, as a measure of population fitness, is a rarely applied but powerful measure to evaluate restoration success (Travis et al., 2002; Ritchie & Krauss, 2012; Kettenring et al., 2014; Williams et al., 2014). Mating system analysis provides an approach to describing genetic variation in future generations (Clegg, 1980; Loveless & Hamrick, 1984). Mating systems describe the mode of transmission of genes from one generation to the
next through sexual reproduction (Clegg, 1980). Breeding system, pollen dispersal limits, availability of pollinators and the biology of the plant can determine the mating system (Barrett et al., 1996; Goldingay & Carthew, 1998; Eckert et al., 2010). Plants exhibit a diversity of mating system from complete selfing to complete outcrossing (Ramsey & Vaughton, 1991; Goodwillie et al., 2005; Heliyanto et al., 2005; Coates et al., 2007), the level of which determine future genetic variation, fitness, and spatial genetic structure (Slatkin, 1985). Inbreeding leads to the reduction in the frequency of heterozygotes, can occur through self-fertilization in self-compatible species, and mating between neighbouring plants with shared ancestors (bi-parental inbreeding) (Charlesworth & Charlesworth, 1987; Lynch, 1991; Ritland, 1996). Subsequent inbreeding depression (the reduced fitness of inbred individuals relative to more outbred individuals) and reduced genetic diversity may occur if this is maintained over multiple generations (Charlesworth & Charlesworth, 1987; Lynch, 1991).

Plant populations in general do not display random mating because they are dependent on pollen vectors and are typically structured genetically due to the combined factors of seed and pollen dispersal and natural selection (Vekemans & Hardy, 2004). Changes in mating systems can have significant implications for the survival and persistence of small populations, as a result of limited pollinator services and/or changes in pollen flow patterns (Ellstrand & Elam, 1993a; England et al., 2001) and reduced seed set can result.

**Pollen dispersal and paternity assignment**

Pollen dispersal influences, and is influenced by, population genetic structure, diversity and fitness and the genetic makeup and vigor of populations (Ouborg et al., 1999; Ghazoul, 2005; Sork & Smouse, 2006). Paternity analysis is established on the identification of the paternal parent of seed progeny from known maternal trees and compares separate alleles in the parental and progeny groups (Ouborg et al., 1999). Indirect approaches can be used calculating the paternal contribution to progeny groups to estimate pollen pool neighbourhoods and pollen dispersal distances (Smouse et al., 2001; Austerlitz & Smouse, 2002; Robledo-Arnuncio et al., 2006). These techniques allow us to determine gene flow through pollen dispersal to evaluate restoration genetic integration and contemporary mating patterns (Broadhurst et al., 2006; Ashley, 2010; Williams et al., 2014).
**Spatial genetic structure of restored populations**

In natural plant populations spatial genetic structure arises due to the nature of seed dispersal, and especially with primarily gravity dispersed seed (offspring growing in close proximity their maternal trees). In restored populations, however, broadcasting of seed or planting of green stock can mean that spatial genetic structure is not established. This randomising effect can reduce bi-parental inbreeding, as pollen dispersal is occurring between trees that are unrelated (Ritchie & Krauss, 2012). It may also have implications for population fitness (De Cauwer et al., 2010) as effectively random mating is an outcome, even with nearest neighbor pollination. The effect on restoration success of this randomization of genetic structure in restored populations is yet to be thoroughly considered.

Pollen dispersal contributes to this spatial genetic structure, typically reflective of pollinator behaviour (Hirao et al., 2006; Breed et al., 2012a; Phillips et al., 2014). The relative abundance of these pollinators, as a key parameter to measure the restoration success, could have significant impact on the pattern (distance) of pollen flow, even if the species is completely outcrossing, and no bi-parental inbreeding exists within the population. The pattern of pollen flow is directly related to pollinator behaviour, species type and relative abundance of pollinators. Therefore, it is necessary to review pollinator mutualisms and their effect on mating systems when the goal is to restore sustainable populations (Neal, 1998; England et al., 2001; Menz et al., 2011).

**This study**

This study is focused in Southwest Western Australia, a biodiversity hotspot (Myers et al., 2000; Hopper & Gioia, 2004) that is highly fragmented and disturbed. The > 8000 plant species, in the region have been severely impacted, as 82% of the original plant cover has been cleared since European settlement in the early nineteenth century (Beard, 1990). Only 35% of the Banksia woodlands on the Swan Coastal Plain remain and the decline has been most marked in the last 20 years. The ecological restoration of Banksia woodlands has been occurring within sites across Southwest Western Australia for >20 years and many of these sites are now reproductively mature. The restoration industry within the Southwest is setting world-class benchmarks on achieving the re-establishment of diverse, rich, plant species restoration (Petroleum, 2011). These
restoration sites now provide a unique opportunity to ecologically and genetically evaluate the application of restoration practice and guidelines. In particular, they offer a powerful opportunity to assess key issues of genetic pattern and process in a restoration context, with regards to genetic diversity of current and future generations of restored populations. The main objective of my research is to conduct an ecological and genetic assessment of restoration success by assessing levels of population genetic diversity and its spatial structure, mating patterns, and the delivery and diversity of pollinator communities, in restored Banksia woodland sites.

I will assess and compare these measurements within restored populations and their offspring to those of adjacent, fragmented and continuous natural sites within the region of the Swan Coastal Plain. Through this ecological and genetic assessment focusing on two keystone Banksia species, I aim to achieve an insight into the functionality of these restored populations and a measure of their long-term viability.

Specific aims of this research are to:

1. Introduce the study system of Banksia woodlands, presenting background information on each study site and sampling methodologies employed (Chapter 2)
2. Assess the effect of restoration on the diversity, abundance and behaviour of bird and insect pollinator communities (Chapter 3)
3. Assess the impact of pollinator behaviour on realised pollen dispersal, genetic landscape connectivity and reproductive output in B. menziesii (Chapter 4)
4. Quantify the success of restoration of genetic diversity and spatial genetic structure within restored B. menziesii populations (Chapter 5)
5. Assess the effects of high and low quality restoration approaches for genetic integration, connectivity and mating systems of B. attenuata (Chapter 6)
6. Summarise the knowledge gained and address the implications this research for restoration practitioners and regulators. Potential further research and directions that have arisen from this work are also discussed (Chapter 7).

Few studies have genetically evaluated restoration success. This study is one of the first to specifically assess the restoration of reproductive functionality. It will provide a solid genetic basis for future restoration and conservation work to better understand the
mechanisms underpinning mating systems and pollinator mutualisms in a biologically diverse ecosystem under ongoing and increasing anthropogenic disruption.
Chapter 2: Banksia woodland restoration: study sites, species and landscape context

Destruction and restoration of Southwest Western Australian biodiversity

In Australia, broad-scale land clearing has occurred since European settlement. In Western Australia, land clearance for agriculture and urbanization are the primary cause for loss of habitat and urban development continues to shrink and fragment the remaining native vegetation. Ecological restoration is a primary action to address the detrimental consequences of land clearing and habitat degradation. However, an understanding of these natural systems, essential to underpin the conservation and restoration management of this landscape, is limited (Lindenmayer et al., 2008; Phillips et al., 2010). Reference sites are used in ecological restoration for (i) goals for restoration; (ii) providing templates to which restored sites can be designed; and (iii) establishing a framework for post-restoration monitoring (Brinson & Rheinhardt, 1996; SER, 2004). Remnants or fragments of historical natural areas are often candidates for reference sites (White & Walker, 1997; Rheinhardt et al., 1999; SER, 2004; Williams, 2011). However, the definition of ‘naturalness’ can be difficult in some situations given the widespread nature and extent of disturbance, as reference sites can be degraded in some way (Hobbs & Norton, 1996; Williams, 2011).

The current research was conducted in Banksia woodland, a defining woodland community of the Swan Coastal Plain Bioregion (SCP) (Fig. 2.1). The SCP lies along the Indian Ocean, within the Southwest Australian Florisitic Region (SWAFR), one of 25 global biodiversity hotspots (Myers et al., 2000; Hopper & Gioia, 2004). The SWAFR region covers only 13% of the land mass of Western Australia, yet with ca. 8000 species, contains 75% of the states vascular plant species. Approximately 15% of these species are pollinated by vertebrates, the highest record in the world (Keighery, 1980). Over 2,250 invertebrate species have been recorded for the region (Knowles, 2011).

These Banksia woodlands are dominated by small trees (4-12m) that are restricted to the Mediterranean climate region of southwest Western Australia (Hopper & Burbidge, 1989). Once extensive in distribution (more than 6,000 km²), by 1986, 55% of the
Banksia woodlands had been cleared (Hopper & Burbidge, 1989) and now only 35% of the woodlands remain, with only 7% in conservation reserves (Lamont et al., 2007), due to development or clearing for urban use (Fig. 2.2).

A large proportion of the total water usage (~70%) by metropolitan Perth is obtained from groundwater resources (Davidson, 1995). The Gnangara Groundwater Mound is the larger of two shallow aquifers from which water is abstracted. This abstraction lowers the water table and has detrimental impacts on ecosystems dependent on shallow groundwater (Kite & Webster, 1989), such as for Banksia woodlands (Groom et al., 2001). Long-term declines in groundwater levels resulting from below average rainfall (low groundwater recharge) and the cumulative effects of abstraction are the primary cause for declines in vigour and distribution of groundwater dependent species such as Banksia ilicifolia and the replacement of B. attenuata with more tolerant B. menziesii (Muir, 1983; Groom et al., 2000). Perth is projected to experience up to 20% less winter rainfall with summer rainfall increasing or decreasing by 10% by 2070 (Groom et al., 2001). Future changes in groundwater recharge and with an expanding demand for water with urban population growth, decline in of these groundwater dependent overstorey species is predicted (Yates et al., 2010).

Another major threatening process for the diverse flora of the SWAFR is the introduced soilborun multi-host plant pathogen Phytophthora cinnamomi (Hill et al., 1994; Shearer & Dillon, 1996; Shearer et al., 2007). Phytophthora cinnamomi is a relatively recent invader into the SWAFR, and Banksia woodlands are vulnerable to infestation with resulting plant death (Shearer & Dillon, 1996).

Due to these threats, functional assessments within this study were conducted on multiple Banksia woodland sites, 10 in total, to capture the range of ‘naturalness’ of what are potential reference sites. Further, fragment shape has been found to contribute to differences in pollinator composition as well as pollen movement, reproduction fitness and viability of Australian plant species, reinforcing the need to study multiple fragmented populations for the range of ‘natural’ reference (Elliott et al., 2012; Llorens et al., 2013). The conditions of these reference sites were analysed throughout this study to determine the highest level of functioning across a suite of functions.
Figure 2.2 Changes in vegetation cover in northern Perth between 1992 and 2013. Images contain study areas (Alexander Park, Highview Park, Marangaroo Conservation Area and Paloma Park) (modified vegetation change maps accessed from Land Monitor Western Australia, Land Information Authority–Landgate, accessed Feb 2014: http://landmonitor-beta.langate.wa.gov.au).
Table 2.1 Proportion of Western Australian terrestrial fauna within Banksia woodlands (Knowles, 2011). Sourced from Banksia Woodland Symposium Proceedings 2011

<table>
<thead>
<tr>
<th>Faunal Category</th>
<th>Percentage of Western Australia species found within Banksia woodlands</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insects</td>
<td>79.6%</td>
</tr>
<tr>
<td>Chelicerates (Spiders, Scorpions, Ticks, Mites, Harvestmen)</td>
<td>15.9%</td>
</tr>
<tr>
<td>Myriapids (Centipedes and Millipedes)</td>
<td>1.6%</td>
</tr>
<tr>
<td>Mammals</td>
<td>0.3%</td>
</tr>
<tr>
<td>Amphibians</td>
<td>0.1%</td>
</tr>
<tr>
<td>Birds</td>
<td>0.8%</td>
</tr>
<tr>
<td>Reptiles</td>
<td>0.8%</td>
</tr>
</tbody>
</table>

Keystone species

Keystone species are categorized by the effects of their removal from a system (Mills et al., 1993). This is recognition that one species can have a disproportionate effect on associated species and ecosystem processes (Power & Scott, 1995), and removal of this species can have a significant impact on ecosystem function (Lambeck, 1999). Mills et al. (1993) categorized the effects of the removal of a keystone plant species as the “extirpation of dependent animals, potentially including pollinators and seed dispersers”. Further to this, there are sub-categories of keystone species, one of which is ‘keystone hosts’ or ‘mutualists’. This group contains plant species that support generalist pollinators, providing critical resources such as nectar to vertebrate species, for example (Mills et al., 1993). The study species for the current study, Banksia attenuata and Banksia menziesii, fall into this category. Removal of these species from Banksia woodlands would greatly impact pollinators and ecosystem functioning. For example, Banksia menziesii is one of the major sources of floral nectar for honeyeaters during winter months (Ramsay, 1989). Investigating, determining and labeling species as ‘keystone’ insists on an interactive, multi-species perspective (Paine, 1995) particularly within restoration targets.
**Banksia attenuata and Banksia menziesii**

**Biology and ecology**

*Banksia* is an endemic Australian genus of sclerophyllous, hermaphroditic plants containing 169 species (Collins & George, 2009; MAST et al., 2012). This region contains 80% of *Banksia* species (Proteaceae; (Thiele & Ladiges, 1996)) of which 64 are restricted to the Mediterranean climate region that defines the bounds of south-western Australia (Lamont et al., 2007). *Banksia* is generally the most prominent taxonomic group in the species rich sandplain flora of Southwest Western Australia (Lamont et al., 2007). Where populations have been fragmented, seed production per plant has also been noted to decline (Lamont et al., 2007). The groups most vulnerable are those that are non-sprouters, rather than resprouters (those that can vegetatively recover post-fire). *Banksia* store seeds within the crown of the plant (scrutiny), which maximizes the number of seeds available for the next generation by protecting them within infructescence (cones) from granivores, agents of decay and fire (Cowling et al., 1987; Lamont et al., 1991; Barrett et al., 2005). Seed release and germination are closely related to fire, and the likelihood of recruitment and survival of any seeds releases in the absence of fires is generally low (Lamont & Enright, 2000).

*Banksia attenuata* (candlestick or slender *Banksia*) and *B. menziesii* (firewood *Banksia*) are widespread tree or woody shrub species, distributed across the Swan Coastal Plain of Western Australia, on deep sand in kwongan, shrubland and woodlands (George & Gardner, 1981; Corrick & Fuhrer, 2002). As these two co-occurring species have such a large distribution over the Swan Coastal Plain, they are used extensively in ecological restoration of sand quarries in the region (Rokich et al., 2002; Rokich & Dixon, 2007).
Banksia attenuata is a tree up to 12m tall (Fig. 2.3) and 0.4 – 2m in a shrub form. It has about 1640-2015 brilliant yellow sessile florets forming inflorescences, arranged orthogonally around a central woody axis forming flower spikes up to 5 cm wide and up to 25-30cm long (George & Gardner, 1981; Wooller et al., 1983; Cowling et al., 1987) (Fig. 2.4). Prior to floret opening, pollen is deposited from sessile anthers onto a modified pollen presenter style-end, directly below the terminal stigmatic groove. After floret opening the style protrudes beyond the relaxed perianth (George 1981). Floret opening on inflorescences (anthesis) proceeds acropetally (maturing from the base upwards) over 10 to 20 days and is asynchronous. The inflorescences are produced over several weeks from spring to late summer (November to February), and thus have different stages of development over the flowering season (Wooller & Wooller, 2001). Overtime, the individual florets fade to brown and grey and oval follicles from fertilized ovules develop maturing over 7-8 months about February to December, with seed maturation from September to December (Stock et al., 1991). Seed set is extremely low, with only 0.1% of florets developing into seed (Cowling et al., 1987). The species is highly serotinous, holding viable seed in the canopy for at least 10 years (Cowling et al., 1987) and is a re-sprouter after fire (George & Gardner, 1981).
Banksia menziesii is a tree up to 10m tall (Fig. 2.3) and 1-3m in a shrub form. It has about 600-1400 sessile florets ranging in colour from yellow, pink to red (37-71mm long) and arranged orthogonally around a central woody axis, forming inflorescences 4-12cm long (Whelan & Burbidge, 1980; George & Gardner, 1981; Ramsey, 1988a) (Figure 2.4). Prior to floret opening, pollen is deposited from sessile anthers onto a modified pollen presenter style-end, directly below the terminal stigmatic groove. After floret opening the style protrudes beyond the relaxed perianth (George 1981). Floret opening proceeds acropetally, with 30-70 opening per 24 hours (Ramsey, 1988a). Of these, approximately 95% open during daytime, mostly in response to foraging by nectivorous birds. By dusk, all of the pollen has been removed from florets suggesting nocturnal pollination is unlikely (Ramsey 1986, 1987). The inflorescences are produced over several weeks in autumn and winter (April to July). The first sets of inflorescences produced are usually yellower and lighter in colour and after colder weather and further into winter they are usually a deeper red (Collins et al., 2009). The species is weakly serotinous (Cowling et al. 1987) and is also a re-sprouter after fire (George 1981).

Both species co-dominate the Banksia woodlands of the SCP, in a range of topographical positions and are known to be groundwater dependent at depths of 6-7m (Dodd & Bell, 1993), but cannot access groundwater at deeper depths of >10m (Farrington et al., 1989). Both have mixed generalist pollinator mutualisms, providing an important source of nectar for a wide range of nectar-feeding birds (predominantly honeyeaters in the family Meliphagidae), native bees and wasps and introduced honeybees Apis mellifera. Both species are also pollinated by small marsupials (Collins & Rebelo, 1987), however marsupial pollination has declined substantially, especially in the metropolitan area, where honey possums are now rare (Sumner et al., 2005). Invertebrates play an important role in pollination of these species, even though they are dominated by, and conform to, bird pollination syndromes (Whelan & Burbidge, 1980; Lewis & Bell, 1981; Ramsey, 1988b). Banksia menziesii are also pollinated by staphylinid beetles (Coleoptera: Staphylinidae) (Ramsey, 1988b), and similar seed set was observed in B. attenuata after cage experiments excluded birds (Whelan & Burbidge, 1980), indicating insects play a role in pollination of these banksias.
Study sites

I selected eight reference bushland sites; four fragments, two within large continuous woodlands, and two fragments adjacent to two restored sites within the study area on the Swan Coastal Plain (Fig. 2.5). All sites were between 5 ha and greater than 750 ha in size with a 50 km distance between the northernmost and southernmost sites. Sites were chosen to represent indicative reserve sizes, shapes and internal characteristics of a reference system to capture the range of ‘naturalness’ of what are potential reference sites for restoration evaluation. For effective replication, sites were restricted to locations with matched vegetation types, woodlands containing vegetation that was predominantly low, open-woodland with 4-12m *Banksia menziesii* and *Banksia attenuata* trees as co-dominants with a diverse sclerophyllous understory. Due to environmental limitations, the experimental design is unbalanced as there were no other *Banksia* woodland restoration sites that contain both species in a reproductive stage of development (other than one within an active mining pit studied by Frick et al. 2014). To calculate gene flow between restored and natural sites, adjacent natural populations were studied, similarly adjacent fragmented sites of high and low quality were chosen for genetic studies.
Figure 2.4 *Banksia attenuata* (above) and *Banksia menziesii* (left) of inflorescences to infructescence (cone) succession stages. Photographs taken by author.
Figure 2.5 Ten study site locations on the Swan Coastal Plain, Western Australia; A: NN, Neaves North, NS, Neaves South; B: GN, Gnangara natural, GR, Gnangara restored; C: AP, Alexander Park, HP, Highview Park, PP, Paloma Park, MC, Marangaroo Conservation Area; D: JN, Jandakot natural, JR, Jandakot restored.
Continuous Banksia woodland

Neaves North – NN and Neaves South – NS (Fig. 2.5A; 2.6)

Within continuous (>750 ha) Banksia woodland, these two sites (Neaves North and Neaves South) are located north and south of Neaves Road in Melaleuca, Western Australia, approximately 50km north of Perth. These sites occur on the Gnangara Groundwater Mound, on the Bassendean sand dune system. The vegetation is of Bassendean Complex- North and Yanga Complex, largely determined by the underlying landforms, depth to water table, soils and climatic conditions (Groom et al., 2001). The site consists of low open woodlands of Banksia attenuata and Banksia menziesii with dominant shrubland species Verticordia nitens, Adenanthes cygnorum, Xanthorrhoea preissii, Dasypogon bromelifolius, Melaleuca systena, Macrozamia riedlei and Beaufortia elegans. The sites have low disturbance, with few weeds. Drawdown of the Gnangara Groundwater Mound for Perth’s water supply has been studied as the cause for the sites declining in site health (Groom et al., 2001) (Table 2.3).

Figure 2.6 Continuous Banksia woodland showing sparse arrangement of overstorey trees. Photograph taken in the middle of the site, by author.
Fragmented Banksia woodland

Alexander Park – AP (now known as Hepburn Park) (Fig. 2.5C; 2.7)

Alexander Park is situated approximately 16 kilometres north of the city of Perth. Located on the Spearwood Dune system, the site was fragmented from larger woodland around 1998. The vegetation community consists of low woodlands to low open woodlands of *Banksia attenuata*, *B. menziesii* and *B. ilicifolia* over species-rich dense shrubland. The dominant shrubland species are *Beaufortia elegans*, *Leucopogon polymorphus*, *Leucopogon conostephioides*, *Melaleuca systena*, *Calytrix angulata*, *C. flavescens*, *Stirlingia latifolia*, *Dasypogon bromeliifolius*, *Lyginia barbata*, *Macrozamia riedlei* and *Xanthorrhoea preissii*. The Park is very species rich and in good condition, and contains over 128 plant species (Table 2.3). One large track existed through the middle of the site but now is overgrown.
Highview Park – HP (Fig. 2.5C; 2.8)

Highview Park is situated approximately 16 kilometres north of the city of Perth and was fragmented from larger woodland around 1998. Located on the Bassendean Dune System, it consists of Bassendean complex vegetation. The vegetation structure is somewhat intact, with the overstorey containing Banksia attenuata and B. menziesii, sometimes with Eucalyptus todtiana and E. marginata. The dominant shrubland species are Adenanthos cygnorum, Xanthorrhoea preisii, Ermaea pauciflora, Hibbertia huegelii, H. hypericoides and species of Leucopogon. There are six dissecting sand tracks through this reserve with high number of weeds dominating tracks and understorey edges. Attached to a recreation area, there is a lot of dumped rubbish, human traffic, and illegal tracks made by four-wheel drive vehicles, trail bikes and the public camping.

Figure 2.8 Highview Park Banksia woodland showing tree deaths and disturbance. Photograph taken in the middle of the site, by author.
Paloma Park – PP (Fig. 2.5C; 2.9)

Paloma Park is situated approximately 15 kilometres north of the city of Perth, and became fragmented from larger woodland around 1996. Located on the Spearwood/Bassendean Dune system, its vegetation consists of Karrakatta Central and South complex. The vegetation structure in this small disjunct reserve is moderately intact, with the overstorey dominated by *Banksia attenuata*, containing *B. menziesii* and with few *Eucalyptus marginata* over a very diverse and species rich shrub understorey. The dominant shrubland species are *Conostephium pendulum*, *Hibbertia huegelii*, *H. hypericoides*, *Petrophile linearis* among other shrub species. There are a few management issues with this reserve including weed control, rubbish dumping, spot fires and access tracks, three of which dissect the reserve and increase edge effects. Restoration plantings of reproductively active *B. menziesii* are present on the borders of the reserve.

Figure 2.9 Paloma Park *Banksia* woodland showing disturbance. Photograph taken in the middle of the site, by author.
**Marangaroo Conservation Area – MC (Fig. 2.5C; 2.10)**

The Marangaroo Conservation Area site is situated approximately 15 kilometres north of the city of Perth, and became fragmented from larger woodland around 1998. Located on Spearwood Dune system, it consists of Karrakatta Central and South complex vegetation. It contains low open forest to low open woodlands of *Eucalyptus todtiana* and *E. marginata* over *Banksia attenuata* and *B. menziesii*. The dominant shrubland species are *Adenanthos cygnorum*, *Leucopogon polymorphus*, *Calytrix angulata*, *C. flavescens*, *Stirlingia latifolia*, *Dasypogon bromeliifolius*, *Leucopogon*, *conostephioides*, *Lyginia barbata*, *Macrozamia riedlei* and *Xanthorrhoea preissii*. It is a long linear fragment adjacent to a golf course, containing trees of poor health (Table 2.3), perhaps due to increased nutrient runoff from the golf course. Excessive nutrients can be harmful to these poor-nutrient adapted plant communities (Grigg et al., 2000). The fragment contains one main track running north–south and three perpendicular tracks west-east, however there are visibly low edge effects, and few weeds.

Figure 2.10 Marangaroo Conservation Area *Banksia* woodland showing tracks and disturbance. Photograph taken in the middle of the site, by author.
Natural Banksia woodland sites adjacent to restored sites

Gnangara natural – GN (Fig. 2.5B; 2.11)

The Gnangara natural site is situated approximately 40 kilometres north of the city of Perth. It is situated adjacent to the Gnangara restored Banksia woodland. It is located on Bassendean Dune system occurring on the Gnangara Groundwater Mound containing Bassendean-North complex vegetation. This site contains taller open woodlands with Banksia attenuata and B. menziesii dominant, with B. ilicifolia, Eucalyptus todtiana, E. marginata and Nuytsia floribunda. The dominant shrubland species are Adenantheros cygnorum, Xanthorrhoea preissii, Eremaea pauciflora and Leucopogon species. Lowering of the Gnangara Groundwater Mound is thought to be the cause of the decrease in plant health on these uplands.

Figure 2.11 Gnangara natural, adjacent Banksia woodland to Gnangara restored site. Photograph taken in the middle of the site, by author.
Jandakot natural – JN (Fig. 2.5D; 2.12)

Jandakot natural site is situated approximately 21 kilometres south of the city of Perth. It is a natural site that is linked to Jandakot Regional Park, located on Bassendean/Spearwood Dune system containing a Bassendean-Central and South vegetation complex, occurring on the Jandakot Groundwater Mound. The site is rich in diversity and complexity, the low woodland overstorey contains Banksia attenuata, B. menziesii, B. ilicifolia, B. grandis, B. littoralis, Eucalyptus todtiana, E. marginata and Nuysia floribunda, and with a dense understorey of sclerophyll shrubs. The dominant shrubland species are; Daviesia quadrilatera, Pimelea sulphurea, Eremaea pauciflora, Jacksonia floribunda, Scholtzia involucrata, Melaleuca scabra, Astroloma xerophyllum, Eremaea pauciflora, Stirlingia latifolia, Xanthorrhoea preissii, Calothamnus sanguineus, Conospermum stoechadis and Hypocalymma angustifolium. This site also contains a mixed shrub damplands, a seasonal wetland area, containing Eucalyptus rudis and Melaleuca preissiana over open heath of Regelia cilata, Calothamnus lateralis, Lyginia imberbis. Areas of localised disturbance contain weed species, but overall the vegetation condition of this site is excellent.
Figure 2.12 Jandakot natural, adjacent *Banksia* woodland to Jandakot restored site, including Malaise Trap. Photograph taken in the middle of the site, by author.

**Restored Banksia woodland**

The landscape of restored sites are vastly changed with the removal of desired sand layers, the topsoil and organic matter with approximate depth of 150mm is stripped off and kept till re-vegetation (Fig. 2.13). Within these post-mined/sand-extracted sites, the return of the topsoil seedbank is considered to be the most practical means of plant replacement, with the use of seed broadcasting, planting seedlings or mulch/brush application provide a means to supplement plant numbers at a site (Rokich & Dixon, 2007).
Gnangara restored – GR (Fig. 2.5B; 2.14)

Gnangara restored site situated approximately 40 kilometres north-northeast of Perth, and is located within a Rocla Quarry Products leasehold, a post-sand mine restored site planted in 1996. The vegetation was restored to site via top-soil replacement, broadcasting seed and green-stock planting on a lowered landscape. The rehabilitation efforts at Rocla satisfied completion criteria with the focus on rehabilitating species richness and plant density and cover. Details of the restoration techniques employed can be found in Rokich and Dixon (2007).
Figure 2.14 Gnangara restored Banksia woodland. Photograph taken in the middle of the 1996 restored site, by author.

**Jandakot restored – JR (Fig. 2.5D; 2.15)**

Jandakot restored site is situated approximately 21 kilometres south of the city of Perth, within a post-sand-extraction site within a previously owned 57-hectare Rocla Quarry Products leasehold. The plants in the restored population are roughly 16 years old with rehabilitation completed in 1995. The initial planning for rehabilitation efforts at Rocla satisfied completion criteria (of 1990’s standard), however according to the Final Landform Plan of 1995 (Rocla Quarry Products 1995), the objectives outlined an uncertain future for the rehabilitation program due to the longer-term outlook of changed land use for the site i.e. residential or industrial use (Rocla Quarry Products 1995). Therefore this site lacks natural structure in overstorey but chiefly in the understorey. Much of the site contains no herbaceous understorey, containing only bare sand and weed species.
Field Sampling Techniques

Population health

To identify levels of disturbance and fragmentation, bushland health surveys were conducted at each site with methods adapted from Hnatiuk et al. (2009). Each *Banksia menziesii* tree at each site was surveyed and categorized into one of the following growth stages (Figure 2.16; Table 2.1). As *B. menziesii* provides the main source of nectar during winter months (Ramsey 1989), is believed to be changing in dominance within woodlands due to reduced access to ground water (Groom et al., 2001) and is the main focus of the genetic studies within this thesis (Table 2.4), health surveys not carried out on *B. attenuata.*
Figure 2.16 Growth stages from (Hnatiuk et al., 2009).

Table 2.2 Indicators of growth stage from (Hnatiuk et al., 2009).

<table>
<thead>
<tr>
<th>Code</th>
<th>Growth stage</th>
<th>Trees dominant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Early regeneration</td>
<td>Dominated by small, juvenile des to very sparse regenerating plants, with or without a few older, widely spaced, emergent plants.</td>
</tr>
<tr>
<td>2</td>
<td>Advanced regeneration</td>
<td>Dominated by dense to sparse, well-developed, immature plants. Large emergents may be present with crown cover less than 5 per cent of the total crown cover. If the cover is more than 5 per cent, however, classify as ‘uneven age’. Trees have well-developed stems (poles). Crowns have small branches. The height is below maximum height for the stand type. Apical dominance still apparent in vigorous trees.</td>
</tr>
<tr>
<td>4</td>
<td>Uneven age</td>
<td>Mixed size and age classes, usually identified by two or more strata dominated by the same species, but can also include sites with different species regenerating in the understorey of an older canopy.</td>
</tr>
<tr>
<td>4</td>
<td>Mature</td>
<td>Well-spaced mature-sized plants or densely packed plants with crown touching, with or without emergent senescent plants. Trees at maximum height for the type and conditions. Crown at full lateral development in unlocked strand. No apical dominance.</td>
</tr>
<tr>
<td>5</td>
<td>Senescent</td>
<td>Dominated by over-mature plants, particularly in the dominant stratum; evidence of senescence in many plants, some without obvious links to disturbance. Tree crowns show signs of contracting dead branches and decreased crown diameter and leaf area. Distorted branches and burls may be common. Dead trees may be present.</td>
</tr>
</tbody>
</table>
Figure 2.17 Principal components analysis (PCA) plot of measured environmental variables (Table 2.3) using Euclidean distances, characterizing study sites of *Banksia menziesii* along the Swan Coastal Plain. Created in PRIMER v 6 (Clarke, 1993) AP, Alexander Park; HP, Highview Park; MC, Marangaroo Conservation Area; PP, Paloma Park; NN, Neaves North; NS, Neaves South; GN, Gnangara natural; GR; Gnangara restored; JN, Jandakot natural and JR; Jandakot restored.
Table 2.3 Site characteristics and measurements of *Banksia menziesii* tree plant density, average diameter at breast height (DBH), height, health, and growth stages within each study area. Confidence intervals are shown in parentheses.

<table>
<thead>
<tr>
<th>Population (Latitude, Longitude)</th>
<th>Code</th>
<th>Type</th>
<th>Area Size surveyed (ha)</th>
<th>Isolation (km)</th>
<th>Density (plants/ha)</th>
<th>Mean DBH (cm)</th>
<th>Mean Height (m)</th>
<th>% Growth stages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neaves North (31 40’28”S, 115 53’44”E)</td>
<td>NN</td>
<td>Natural</td>
<td>&gt;750</td>
<td>-</td>
<td>19.1</td>
<td>13.2 (12.2-14.2)</td>
<td>3.6 (1.5-5.7)</td>
<td>87</td>
</tr>
<tr>
<td>Neaves South (31 41’11”S, 115 53’50”E)</td>
<td>NS</td>
<td>Natural</td>
<td>&gt;750</td>
<td>5.07</td>
<td>-</td>
<td>20.1</td>
<td>13.0 (12.6-13.4)</td>
<td>3.2 (2.0-4.4)</td>
</tr>
<tr>
<td>Alexander Park (31 49’09”S, 115 52’29”E)</td>
<td>AP</td>
<td>Fragment</td>
<td>9.8</td>
<td>4.2</td>
<td>0.81</td>
<td>118.6</td>
<td>15.9 (15.5-16.4)</td>
<td>3.6 (1.8-5.4)</td>
</tr>
<tr>
<td>Highview Park (31 49’38”S, 115 51’31”E)</td>
<td>HP</td>
<td>Fragment</td>
<td>10.9</td>
<td>4.9</td>
<td>0.50</td>
<td>42.7</td>
<td>11.3 (10.6-11.9)</td>
<td>3.0 (2.9-3.1)</td>
</tr>
<tr>
<td>Marangaroo Conservation Area (31 49’49”S, 115 50’08”E)</td>
<td>MC</td>
<td>Fragment</td>
<td>22.41</td>
<td>6.77</td>
<td>0.09</td>
<td>115.1</td>
<td>11.3 (10.9-11.7)</td>
<td>2.9 (2.8-3.0)</td>
</tr>
<tr>
<td>Paloma Park (31 49’59”S, 115 51’07”E)</td>
<td>PP</td>
<td>Fragment</td>
<td>5.06</td>
<td>5.06</td>
<td>0.53</td>
<td>73.1</td>
<td>10.3 (9.9-10.6)</td>
<td>2.4 (2.3-2.5)</td>
</tr>
<tr>
<td>Gnangara Natural (31 47’07”S, 115 56’23”E)</td>
<td>GN</td>
<td>Adjacent</td>
<td>18.4</td>
<td>4.7</td>
<td>-</td>
<td>19.1</td>
<td>15.1 (14.8-15.4)</td>
<td>3.8 (2.3-5.3)</td>
</tr>
<tr>
<td>Gnangara Restored (31 47’09”S, 115 56’32”E)</td>
<td>GR</td>
<td>Restored</td>
<td>33.2</td>
<td>4.9</td>
<td>-</td>
<td>90.7</td>
<td>6.9 (6.2-7.6)</td>
<td>2.4 (2.3-2.5)</td>
</tr>
<tr>
<td>Jandakot Natural (32 06’18”S, 115 52’07”E)</td>
<td>JN</td>
<td>Adjacent</td>
<td>14.0</td>
<td>4.1</td>
<td>-</td>
<td>212.9</td>
<td>15.6 (14.6-16.6)</td>
<td>3.7 (2.2-5.2)</td>
</tr>
<tr>
<td>Jandakot Restored (32 06’28”S, 115 52’01”E)</td>
<td>JR</td>
<td>Restored</td>
<td>25.6</td>
<td>4.1</td>
<td>-</td>
<td>78.2</td>
<td>7.7 (6.5-8.9)</td>
<td>2.3 (2.1-2.5)</td>
</tr>
</tbody>
</table>
This study has used a combination of sites for ecological and genetic studies, see table below for details (Table 2.4).

Table 2.4 Sites used for each study and related chapter.

<table>
<thead>
<tr>
<th>Site</th>
<th>Continuous</th>
<th>Fragmented</th>
<th>Adjacent</th>
<th>Restored</th>
</tr>
</thead>
<tbody>
<tr>
<td>Studies</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pollinator observations</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>and collections</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>B. menziesii</em> genetic study</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><em>B. attenuata</em> genetic study</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental Chapter</td>
<td>3,4,5</td>
<td>3,4</td>
<td>3,4,5</td>
<td>3,4</td>
</tr>
</tbody>
</table>
Chapter 3: Plant diversity influences pollinator assemblages and foraging behavior in restored and natural Banksia woodlands.

ABSTRACT

Restoration of reproductive functionality in animal-pollinated plant communities requires pollinators for the delivery of pollinator services. To assess the re-establishment of pollinators within restored Banksia woodland sites, the diversity, foraging and interaction behaviour of birds, and the diversity of Pterygota (flying insects) trapped using Malaise tents, were assessed. These parameters were measured within restored (high and low complexity of restored plant communities) and natural (continuous and fragmented) sites during summer and winter flowering of B. attenuata and B. menziesii, respectively, over three years. Functional diversity and abundance of Pterygota differed between restored and natural sites, and with restored site complexity, with the low complexity restoration site containing lower insect diversity (H’ = 1.64, λ = 0.59) than the high complexity site (H’ = 0.75, λ = 2.67). Although bird pollinators are known to be highly mobile, observed bird diversity and behaviour differed between site and site types, surprisingly across distances of < 200 m between sites. Restored sites had similar pollinator species composition to their adjacent natural sites, suggesting these natural sites aid pollinator re-establishment. Bird richness and abundance was lowest, and European Honeybee (Apis mellifera) visitation highest, in fragmented natural sites. Bird aggression was greatest in restored sites, as larger bodied Western Wattlebirds (Anthochaera chrysoptera) and aggressive New Holland Honeyeaters (Phylidonyris novaehollandiae) dominated nectar resources in winter. Territoriality of these bird species altered pollinator movement patterns and increased intra-tree and near-neighbour foraging. Quantifying species composition, richness and behavior has confirmed the importance of restoring diverse species rich habitat to establish and maintain reproductive functionality.
INTRODUCTION

The pollination of flowering plants by animals represents a critical ecosystem process, with over 85% of angiosperms relying on animal-mediated pollination for reproduction and maintenance of genetic variability while supporting over 300,000 flower-visiting species worldwide (Kearns & Inouye, 1997; Ollerton et al., 2011). There is increasing evidence that habitat alteration and disturbance negatively influence and impact pollinators and plant-pollinator interactions (Eckert et al., 2010; Winfree et al., 2011). Addressing the effects of anthropogenic habitat disturbance on natural systems is particularly challenging as most organisms rely on other groups of species for critical ecosystem services, such as pollination and dispersal (England et al., 2001; Menz et al., 2011).

Ecological restoration programs are being implemented worldwide to address the impacts on disturbed habitats. Ecological restoration is the practice of assisting the recovery of degraded, damaged, or destroyed ecosystems by forming new biological communities, ideally representative of the composition, diversity and functionality of the pre-disturbance habitat (SER, 2004). Ecological restoration has largely focused on plant species richness and habitat structure (Hobbs & Norton, 1996; Dobson et al., 1997; Young, 2000; Williams, 2011). Consequently, restoration success has been typically measured against achievements in these structural properties of ecosystems (Ruiz-Jaén & Aide, 2005b; Ruiz-Jaén & Aide, 2005a).

Vertebrate and invertebrates play diverse roles, such as seed dispersers or pollinators, driving ecosystem functioning across different systems (Didham et al., 1996; Fragoso & Varanda, 2011). However, with few exceptions (Waltz & Wallace Covington, 2004; McIntire et al., 2007; Talley et al., 2007; Forup et al., 2008; Lomov et al., 2010) they are rarely part of conservation goals, let alone targets in ecosystem restoration (Majer, 2009; Munro et al., 2011; Williams, 2011). Although restoration of ecosystem functional aspects, such as plant-pollinator interactions, have been increasingly recommended to evaluate success (Forup & Memmott, 2005), many ‘non-target’ species, such as pollinators, are assumed to passively colonize (Williams, 2011).

Pollination services have been extensively investigated for agricultural services, where about 70% of crop plants world-wide are at least partially dependent on animals for
pollination (Klein et al., 2007; Aizen et al., 2009). Honeybees alone contribute billions to the global economy (Potts et al., 2006), and several have been recorded to be in ‘the global pollination crisis’ (Allen-Wardell et al., 1998; Ghazoul, 2005; Steffan-Dewenter et al., 2005). It is universally recognised that pollinator services are in decline as a result of anthropogenic disturbances, habitat loss, fragmentation and isolation, agricultural intensification, agrochemicals, diseases, parasites, climate change, introduced non-native plants and competition with managed or introduced pollinators (Potts et al., 2006; Winfree et al., 2009; Winfree, 2010; 2011). How these impacts affect the restoration of pollinator services in ecologically restored plant communities has received little attention.

The restoration of pollination services and plant-pollinator interactions have yet to be fully investigated or taken into account in evaluations of restoration success (Lomov et al., 2010; Menz et al., 2011; Williams, 2011), due to feasibility and insufficient research on the effects of restoration management on plant-pollinator interactions (Lomov et al., 2006). These ecosystem processes may not re-establish themselves naturally in communities undergoing restoration management, as pollinators require additional environmental provisions, such as nesting sites or food resources (Forup et al., 2008). These requirements make the study of mobile species, such as pollinators, a useful tool or ecological indicator for comparing and evaluating restored ecosystems to reference ecosystems (O’Connell et al., 2000; Munro et al., 2011).

Pollinator diversity and abundance is affected by increasing habitat disturbance (Steffan-Dewenter & Westphal, 2008). Data from a variety of ecosystems show positive correlations between biological diversity and ecological functioning (Schwartz et al., 2000; Srivastava & Vellend, 2005; Balvanera et al., 2006). Studies of native pollinators in the context of landscape disturbance have established that the level of pollination is correlated with the species richness and abundance of pollinators (Kremen et al., 2002; Klein et al., 2008; Williams & Winfree, 2013). This further emphasises the importance of larger pollinator community establishment in restoration and that species that are not to be reliant on a minimal or single species of pollinator for service providers may be more successful within restoration (Potts et al., 2006).

There have been several studies revealing a relationship between pollination services and distance from natural or semi-natural habitats (Ricketts et al., 2008; Garibaldi et al.,
Chapter 3: Pollinator assemblages

2011; Munro et al., 2011) highlighting the importance of remaining natural habitat for the maintenance of species (Carvalheiro et al., 2010). The presence of natural habitat within landscapes is expected to affect the ability of pollinators to migrate and re-establish in restored sites and affect their success (Dixon, 2009). The biodiversity value of these sites for pollinators has received some attention, but the services that pollinators provide to flowering plants is still relatively unexplored (Potts et al., 2006). Restoration of pollination services by generalist pollinators is possible (Klein et al., 2007; Menz et al., 2011), but how decreased pollinator diversity affects connectivity, functionality and ultimately the long-term success of restored sites is unknown. The long-term outcome of restoration can be strongly influenced by the presence or absence of these pollinator species with their effect on plant reproduction and gene flow (Forup et al., 2008).

A key component of population functionality in outcrossed species is the delivery of robust pollinator services for the minimisation of inbreeding and the production of outbred offspring through effective pollen dispersal (Moeller, 2004). Altered foraging behaviour can result in pollen limitation, reduced seed set and lower outcrossing rates (Ashman et al., 2004; Coates et al., 2007). The foraging behaviour of pollinators largely determines patterns of mating and reproductive success of individual plants (Ford & Paton, 1982); (Levin & Kerster, 1974), as their behaviour directly controls the origin and the amount of pollen deposited on the stigma (Paton, 1982). Restored ecosystem attributes, such as vegetation structure, plant species composition (Aizen & Feinsinger, 1994; Quesada et al., 2003), or low densities of reproductive plants (Cascante et al., 2002), can change pollinator foraging behaviour and flight distances, ultimately reducing plant-pollinator neighbourhoods (Garcia-Robledo 2010). In order to evaluate pollinator services within restored sites, pollinator presence and behaviour needs assessment and comparison to natural sites (Lindell 2008).

There is a deficiency in the knowledge of how to restore pollinator services in restoration projects, particularly in areas where specialist invertebrate and vertebrate pollinators exist, such as regions with globally high biodiversity (Dixon, 2009). The Southwest Australian Floristic Region (SWAFR), is one of the world’s 25 global biodiversity hotspots (Myers et al., 2000; Hopper & Gioia, 2004), and has the highest recorded frequency of bird pollinated species in the world (Phillips et al., 2010). More than 70% of the native vegetation in this region has been removed for agriculture and
urban expansion, thus the importance of investigating these species rich communities is needed for ecological restoration projects before they decrease further.

The SWAFR also contains many families of nectivorous insects, the main families being Colletidae, Halictidae, Thynninidae (Hymenoptera), Buprestidae (Coleoptera) and Bombyliidae (Diptera) (Brown EM et al., 1997). There is a large amount of variation between insect families and their level of foraging specificity (Houston, 2000; Phillips et al., 2010) and the introduced generalist pollinator, *Apis mellifera* is the most abundant insect pollinator. Information on the long distance movements of nectivorous insects in the SWAFR is unknown, and with international studies demonstrating that body size and flight distance can be poorly correlated (Phillips et al., 2010) and relatively correlated (Greenleaf et al, 2007), further research is required.

The nectarivorous birds of the SWAFR are honeyeaters, members of the family Meliphagidae, and are the most abundant passerines in many Australian habitats (Ramsay, 1989; Ramsey & Vaughton, 1991; Yates et al., 2007). These birds are generalists in nature with broad tastes, visiting a large range of plant taxa across genera and families, most frequently visiting dominant Myrtaceae and Proteaceae (Phillips et al., 2010). A certain level of resilience to habitat disturbance is expected as honeyeaters are highly mobile and lack specific plant-pollinator specialization (Yates et al., 2007). However, with the large-scale habitat decline, there are records of decreased honeyeater distributions and declines in abundance (Recher, 1999; Yates et al., 2007).

Fine-scale movements of honeyeaters and insects are determined by a combination of resources, foraging behaviour and social interactions. Nectar is a major source of energy for SWAFR honeyeaters, its availability varies considerably within woodland habitats, being influenced particularly by the distribution of nectar producing plant species and their flowering phenologies (McFarland, 1986). Previous research has shown that honeyeaters forage preferentially, with medium to larger birds dominating nectar resources (Ford 1979; McFarland 1986; Ramsey 1989; Vaughton 1990). This situation arises through interspecific differences in dominance relations and foraging efficiencies (Ford, 1979; Ford & Paton, 1982; McFarland, 1986). Honeyeaters are likely to also cause geitonogamous (within-plant) pollination events, although most bird foraging movements occur between non-neighbouring plants, suggesting substantially longer distance cross-pollination also occurs (Ramsay, 1989; Krauss et al., 2009).
Chapter 3: Pollinator assemblages

Comprehensive field studies have shown that insects routinely visit some species that conform to the bird pollination syndrome (Phillips et al., 2010). Studies in Australian Proteaceae conclude that excluding bird pollinators from inflorescences results in substantially lower fruit set (Ramsey & Vaughton, 1991; Celebrezze & Paton, 2004). The foraging behaviour of the generalist insect pollinator, *Apis mellifera*, which makes infrequent interplant movements (Richardson et al., 2000), limits their effectiveness as pollinators to self-incompatible species (Scott, 1980; Paton, 1993). Understanding pollinator movements in response to nectar resource availability, spatial arrangement of resources and the interactions among pollinator species has practical outcomes for restoration design.

The majority of pollination biology research has focused on specialized pollinator systems (Kessler & Baldwin, 2011), despite generalized pollinator-plant systems being more common (Waser et al., 1996). We are only now beginning to explore the complexities of generalized pollination systems (Kessler & Baldwin, 2011). Plant-pollinator interactions face a high level of variability in time and space (Alarcón et al., 2008; Petanidou et al., 2008). Some genera of honeyeaters undertake extensive movements to feed on spatially and temporally patchy nectar resources, fluctuating in concert with nectar resources (Keast, 1968; Collins et al., 1984). The nature of these movements varies regionally, reflecting the biogeographic variation in nectar-producing communities (Keast, 1968). Consequently, evaluating the provisions of seasonal nectar resources and its suitability to support more diverse pollinator assemblages of restored sites is necessary.

The ultimate goal of most restoration projects is to establish self-sustaining ecosystems, which mirror the community structure and functioning of the reference site (or those prior to disturbance). There is the need to investigate, manage and promote ecosystem functionality in restored sites, by moving the emphasis from plant establishment to considering the restoration of functional community interactions. This study assesses the variability of bird and insect pollinator communities among multiple natural sites, in order to evaluate the restoration of these pollinator communities within restored sites. Consequently, the objective is to assess the delivery of pollinator services to two keystone Banksia woodland species, *B. attenuata* and *B. menziesii*, within restored sites. Specifically, I address the following questions:
(i) Are there seasonal differences between the composition and visitation rates of pollinators among *B. attenuata* and *B. menziesii* in restored and natural sites (continuous and fragmented)?

(ii) Do restored sites have lower species diversity and visitation of pollinators than natural sites (continuous and fragmented)?

(iii) Is the composition of pollinators in restored sites similar to that of neighbouring adjacent natural sites?

(iv) Do the foraging behaviours and/or movement patterns of pollinators differ between restored and natural sites (continuous and fragmented) of *B. attenuata* and *B. menziesii*?

**METHODS**

*Study sites*

This study was conducted in eight natural and two restored *Banksia* woodland sites along the Swan Coastal Plain, within the southwest region of Western Australia. A range of *Banksia* woodland sites were selected to assess the reference landscape for ecological restoration evaluation. The natural sites comprised two patches that were part of widespread woodland, four fragmented remnants within an urban matrix, and two remnants that were adjacent to the two restored sites. The two natural continuous sites were located within an area of 5 Hectares chosen from within the Gnangara-Moore River State Forest, here named Neaves Road North (NN) and Neaves Road South (NS). The four fragmented sites were located within an urban landscape, here named Alexander (now known as Hepburn) Reserve (AP), Highview Park (HP), Marangaroo Conservation Area (MC) and Paloma Park (PP). The two remnants adjacent to the two restored sites were Jandakot natural (JN) and Gnangara natural (GN). Rocla Quarry Products restored the two sites being assessed, Jandakot Restored site (JR) and Gnangara Restored site (GR). Chapter 2 contains the full site descriptions and maps (Fig 2.3, Table 3.1).
**Proteaceous study species**

The Proteaceae has been at the focus of many studies in pollination ecology in Australia due to its dominance in the landscape (Whelan & Burbidge, 1980; Collins & Rebelo, 1987; Ramsey, 1988b; Ramsay, 1989; Ramsey & Vaughton, 1991). *Banksia attenuata* and *Banksia menziesii* are widespread tree or woody shrub species, distributed across the Swan Coastal Plain of Western Australia, on deep sand in kwongan, shrubland and woodlands (George & Gardner, 1981; Corrick & Fuhrer, 2002). These two co-occurring species have a wide distribution and are often referred to as keystone species or framework plants, as they are species that provide significant nectar or pollen resources to a large number of pollinators (Menz et al., 2011). Both species are used extensively in ecological restoration of sand quarries in the region (Rokich & Dixon, 2007).

*Banksia menziesii* and *B. attenuata* are highly dependent on animal pollinators for pollination due to their obligately outcrossing breeding system (Scott 1980) and it is vital that these mutualistic relationships are re-established in restoration. Generalist animal and insect species service both species. *Banksia menziesii* flowers during autumn/winter (April-August) and is most likely the major source of floral nectar for honeyeaters (Meliphagidae) during the winter months (Ramsay, 1989). *Banksia attenuata* flowers during summer (November – February) and also attracts a higher number of pollinating insects (Scott, 1980; Wooller & Wooller, 2001). Historically, small marsupial species such as honey possums (*Tarsipes rostratus*), provided pollination services. However, they are now absent from urban *Banksia* woodland sites and highly unlikely to naturally recolonize (Sumner, 2005) (See chapter 2 for more detailed descriptions of study species). Given the difference in area, age structure and level of disturbance across all sites, an attempt was made to quantify attractiveness of sites to pollinators. Flowering intensity was estimated for 10 trees randomly selected in each study area (and a selected set of sites sampled for genetic analyses Chapter 4, 5 and 6). I counted the number of inflorescences produced per tree each year for both *B. attenuata* and *B. menziesii* (Table 3.1).
Table 3.1 Site characteristics and mean inflorescence count per tree for 10 trees in each site (95% confidence intervals) for *Banksia attenuata* and *B. menziesii* between 2010 – 2013. No results were recorded for *B. attenuata* flowering in JN and JR for summer 2010/2011.

<table>
<thead>
<tr>
<th>Site</th>
<th>Code</th>
<th>Characteristics</th>
<th>Characteristics</th>
<th>Mean inflorescences per tree</th>
<th>Mean inflorescences per tree</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neaves North (31 40'28&quot;S, 115 53'44&quot;E)</td>
<td>NN</td>
<td>Continuous</td>
<td>Size (ha)</td>
<td>Area surveyed (ha)</td>
<td>6.5</td>
</tr>
<tr>
<td>Neaves South (31 40'09&quot;S, 115 53'49&quot;E)</td>
<td>NS</td>
<td>Continuous</td>
<td>&gt;750</td>
<td></td>
<td>(4.4-8.6)</td>
</tr>
<tr>
<td>Alexander Park (31 49'09&quot;S, 115 52'29&quot;E)</td>
<td>AP</td>
<td>Fragment</td>
<td>9.8</td>
<td>4.2</td>
<td>10.8</td>
</tr>
<tr>
<td>Highview Park (31 49'38&quot;S, 115 51'31&quot;E)</td>
<td>HP</td>
<td>Fragment</td>
<td>10.8</td>
<td>4.9</td>
<td>9.1</td>
</tr>
<tr>
<td>Marangaroo Conservation Area (31 49'44&quot;S, 115 50'09&quot;E)</td>
<td>MC</td>
<td>Fragment</td>
<td>22.4</td>
<td>4.7</td>
<td>8.9</td>
</tr>
<tr>
<td>Paloma Park (31 49'57&quot;S, 115 51'06&quot;E)</td>
<td>PP</td>
<td>Fragment</td>
<td>5.1</td>
<td>5.1</td>
<td>13.6</td>
</tr>
<tr>
<td>Gnangara natural (31 47'07&quot;S, 115 56'23&quot;E)</td>
<td>GN</td>
<td>Adjacent</td>
<td>18.4</td>
<td>4.7</td>
<td>8.4</td>
</tr>
<tr>
<td>Gnangara restored (31 47'09&quot;S, 115 56'32&quot;E)</td>
<td>GR</td>
<td>Restored</td>
<td>33.2</td>
<td>4.9</td>
<td>6.6</td>
</tr>
<tr>
<td>Jandakot natural (32 06'18&quot;S, 115 52'07&quot;E)</td>
<td>JN</td>
<td>Adjacent</td>
<td>14.0</td>
<td>4.1</td>
<td>-</td>
</tr>
<tr>
<td>Jandakot restored (32 06'28&quot;S, 115 52'01&quot;E)</td>
<td>JR</td>
<td>Restored</td>
<td>25.6</td>
<td>4.1</td>
<td>-</td>
</tr>
</tbody>
</table>
Chapter 3: Pollinator assemblages

Insect diversity and abundance

Insect diversity was assessed using three Malaise trap tents were erected within each site adjacent to flowering trees during each flowering season of *Banksia attenuata* (December to February) and *Banksia menziesii* (May to July) (Fig. 3.1, 3.2) during the period pollination observation surveys were carried out. A total of six tents per site per flowering season were used to collect flying terrestrial insects (Pterygota). Vapona (No-Pest® Strip) was used as the killing agent. Malaise traps capture large numbers and a high diversity of Pterygota, including Hymenoptera, and have been widely used in surveys of insect abundance and diversity (Campbell & Husband 2007). All Pterygota collected were classified into general functional groups (decomposer, disperser, flower visitor, generalist, herbivore, larva, nectar/pollen feeder, omnivore, parasitic, predator, sap-sucker or scavenger) and sorted into possible pollen carriers (mainly Hymenoptera) and other non-pollinating flying insects. The potential pollinators of *Banksia* were then identified and classified further into either morphospecies or, where possible, into the lowest taxonomic classification possible.

![Figure 3.1 Diagram of a Malaise Trap tent structure and dimensions. Inset (left) is a photograph of the killing agent, Vapona (yellow cube) and insects captures within the bottle (photographed by author).](image)
Floral visitor observations

Two trees with flowering inflorescences, and their surrounding neighbours, were monitored at each site within each flowering season for *Banksia attenuata* (during the austral summer; November to February) and *Banksia menziesii* (during the austral autumn; April to June) over three years (winter 2010 – summer 2013). Trees were monitored by the same observer (AR) and one volunteer once each season in 2010/2011 and twice each season thereafter, during days without rain or high wind. On each day, visits to inflorescences by insects and bird species were observed for eight, 10-minute census periods starting within 30 minutes of sunrise. Temperatures and weather conditions were also recorded hourly to determine if they were related to the abundance of visitors. Floral visitor abundance was accepted as a proxy for pollination and was previously recognised as a suitable measure (Williams, 2011).
Chapter 3: Pollinator assemblages

Foraging behaviour

Observations of foraging behaviour by birds and honeybees (*Apis mellifera*) were made during the hours of highest activity (0600 - 1600), based on methods used by Ramsay (1989) and Whelan et al. (2009). For each *A. mellifera* visit, surveyors recorded whether the bee was observed removing pollen or nectar or both. For each honeyeater visit, surveyors recorded the species and foraging behaviour as intra-tree, near neighbour and distant *Banksia* tree movement. In addition, displacement and interaction patterns (within and between bird species) were recorded as intraspecific or interspecific. The aggressive interactions were combined across surveys and seasons within each site and displayed as a matrix. Proportional body size was represented to investigate if dominance was linked to body weight. The number and directionality of intraspecific and interspecific chases observed between bird species were displayed in the matrix. This matrix may represent a network-like paradigm, however it is not based on simulation modelling and does not have accompanying statistical tests for strength of relationships (i.e. networks in proceeding Chapters 4 and 6), it is based on direct observations.

DATA ANALYSIS

Measuring biodiversity

For comparison of insect community structure between restored and natural *Banksia* woodlands, four different community indices were determined, species richness (*d*, adjusted for sample size), Shannon index (*H'*), Simpson index (*λ'*), and effective number of species (*H*<sub>SR</sub>). Shannon index increases as both the richness and evenness of the community surveyed increases, providing a simplistic measure to compare sites. Values typically range between 1.5-3.5 in most ecological studies and rarely > 4 (Magurran & Magurran, 1988). The Simpson index is based on the probability of any two individuals drawn at random from an infinitely large community belonging to the same species and is a measure of dominance. For example, as the index increases, diversity (in the sense of evenness) decreases, and thus values are reported between 0 and 1, 1 being the limit of a monoculture. The Shannon index considers two axes of variation, richness and evenness, and therefore the different distributions of abundance for the same set of species, can result in multiple communities with the same value of entropy. The effective number of species was calculated using the conversion of
Shannon index to true diversities \((\text{Exp}(H'))\) (see Jost, 2006) gives indices a set of common behaviour and properties, and is measured in units of number of species (Jost, 2006; Ellison, 2010; Jost et al., 2010).

**Insect and bird assemblages**

The multivariate analyses of bird and insect assemblages between *Banksia* species (season) and site were performed with non-metric multidimensional scaling (NMDS) using PRIMER v 6 (Clarke, 1993). NMDS is a non-linear ordination method based on rank dissimilarities, preserving ordered relationships so that similar objects between sites and species are visualised closer together in each plot (Legendre & Legendre, 1998). This method is sensitive to showing outliers and the distance between points shows the relative similarity (McCune et al., 2002). NMDS analyses were based on Bray-Curtis distances as the measure of ecological dissimilarity, after \(\log(x + 1)\) transformation of species abundances was used. This distance measure emphasises the relative abundance of the species rather than mere presence, and is a particularly useful approach for bird communities which comprise of a small number of species, differing only in their relative dominance in the bird community (Pillsbury et al., 2011; Reynolds & Symes, 2013). NMDS uses an iterative procedure to minimize a property called ‘stress’, which decreases as the rank-order between distance and dissimilarity improves, where stress <10% is considered ‘good’, 10-20% is ‘fair’ and >20% is ‘poor’ (Kruskal, 1964). This graphical framework is a useful technique to evaluate ecological similarity between sites. The data analysis was repeated using presence-absence data, rather than relative abundances, to determine whether any differences among sites were due to species uniqueness, that is whether species unique to restored, fragmented and continuous sites, or to differences in relative abundance of shared species. Bubble plots were used to effectively display individual species abundances in relation to each *Banksia* woodland site.

An analysis of similarity (ANOSIM) was used to determine whether the insect and observed pollinator (insect and bird) assemblages were similar in composition within and between sites. An ANOSIM tests the hypothesis that there are differences in species composition within and between the assigned groups through determining similarities using the constructed Bray-Curtis matrices and running a permutation/randomisation test (applying 1000 permutations) (Clarke, 1993). A \(P\)-value is generated and a positive
test statistic indicates that the groupings are meaningful; samples within groups are more similar than samples from different groups. This method provides a means of assessing whether the classification of sites into type (restored, fragmented, continuous) was meaningful and whether there was any notable differences in species composition between these site types.

Similarity percentages (SIMPER) were calculated to identify the contribution of each functional group of insects (nectar pollen feeders, parasitic and generalists), family level of insects, and observed pollinator species to the observed dissimilarity among the site types. The analysis allows the identification of groups and species that are most influential in creating the observed patterns. *Apis mellifera* were excluded from the analysis of observed pollinator species community structure and composition, to establish which of the bird species made the highest contributions to the average Bray-Curtis similarity in each of the site types.

**RESULTS**

*Floral resources between sites*

All sites were in flower simultaneously during the period of study for each species within each year, however flowering intensity differed. *Banksia attenuata* plants produced significantly more inflorescences in the Jandakot natural (JN) site than the Jandakot restored (JR) site in the summers of 2011/2012 and 2012/2013 (*F* = 0.46 df = 2, *P* = 0.02). In contrast *B. menziesii* plants in JR had significantly higher production of inflorescences in 2011 and 2012 than the natural JN site (*F* = 3.04, df = 2, *P* = 0.01).

*Insect richness and abundance*

Insect collections included seven different Orders, with Diptera and Hymenoptera making up 78% of the total (Table 3.2; Appendix 1). *Apis mellifera* was the most abundant hymenoptera collected, making up 77% of all bees collected. Malaise Traps set during the summer flowering of *B. attenuata* yielded 38.8% more insects than the autumn/winter flowering *B. menziesii*. With the high variability in the data, there was no significant difference in species richness (*t* = 1.88, *P* = 0.07) and abundance (*t* = 1.53, *P* = 0.14) among sites. Both natural continuous sites had comparable indices of richness
and diversity. Overall insect richness and abundance was generally greater in the continuous natural sites (Table 3.2, $d$: NN = 16.77, NS = 16.85) than the natural fragmented sites (AP, HP, MC, PP; range $d$: 6.92-10.90) and the restored sites (GR and JR: $d = 17.54$; 9.60). The lowest overall species richness ($d$) was at Marangaroo Conservation Area (MC, $d = 6.92$), followed by the Jandakot natural (JN, $d = 7.39$) then Jandakot restored (JR, $d = 9.60$). Shannon index of diversity was lowest in adjacent populations (GN and JN) and Jandakot restored however, the Simpson indices indicate that these sites had higher diversity as the probability of randomly sampling two individuals from different species in the sites were low (Table 3.2). The Gnangara restored and adjacent Gnangara natural site produced similar numbers of most insect orders (Table 3.2) and flower visitors (Appendix A2; Table 3.2), though the restored site had greater effective number of species ($H_{SR}$, GR = 14.44, GN = 5.92). Jandakot restored had overall higher species richness and diversity than its adjacent natural site (Table 3.2, JN and JR) containing a notably higher distribution of Hymenoptera. Jandakot restored had 50% lower richness at order level in comparison to Gnangara restored (Table 3.2).
Table 3.2 Species richness for taxa of all insects captured by Malaise Traps and diversity indices for each site. Details of insect floral visitors are listed within the appendices (see also Appendix 1 for more detailed classifications).

<table>
<thead>
<tr>
<th></th>
<th>Natural</th>
<th></th>
<th></th>
<th>Natural</th>
<th></th>
<th></th>
<th>Natural</th>
<th></th>
<th></th>
<th>Natural</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Continuous</td>
<td>Fragmented</td>
<td>Adjacent</td>
<td>Restored</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neaves</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>North</td>
<td>South</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NN</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of Individuals, N</td>
<td>651</td>
<td>573</td>
<td>250</td>
<td>389</td>
<td>182</td>
<td>218</td>
<td>931</td>
<td>505</td>
<td>593</td>
<td>574</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of Species, S</td>
<td>111</td>
<td>112</td>
<td>60</td>
<td>66</td>
<td>37</td>
<td>56</td>
<td>108</td>
<td>47</td>
<td>113</td>
<td>62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted species richness, d</td>
<td>16.97</td>
<td>16.85</td>
<td>10.69</td>
<td>10.90</td>
<td>6.92</td>
<td>10.22</td>
<td>15.65</td>
<td>7.39</td>
<td>17.54</td>
<td>9.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shannon Index, H'</td>
<td>2.72</td>
<td>2.70</td>
<td>2.65</td>
<td>2.03</td>
<td>2.26</td>
<td>2.79</td>
<td>1.78</td>
<td>1.00</td>
<td>2.67</td>
<td>1.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simpson's Index, λ'</td>
<td>0.77</td>
<td>0.76</td>
<td>0.78</td>
<td>0.63</td>
<td>0.78</td>
<td>0.85</td>
<td>0.53</td>
<td>0.32</td>
<td>0.75</td>
<td>0.59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effective number of species, H_s</td>
<td>15.18</td>
<td>14.88</td>
<td>14.15</td>
<td>7.61</td>
<td>9.58</td>
<td>16.28</td>
<td>5.92</td>
<td>2.71</td>
<td>14.44</td>
<td>5.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bees, Ants, Wasps</td>
<td>Hymenoptera</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>91</td>
<td>55</td>
<td>49</td>
<td>33</td>
<td>36</td>
<td>79</td>
<td>38</td>
<td>74</td>
<td>51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beetles</td>
<td>Coleoptera</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>6</td>
<td>8</td>
<td>2</td>
<td>8</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cockroaches</td>
<td>Blattodea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flies</td>
<td>Diptera</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>26</td>
<td>14</td>
<td>18</td>
<td>13</td>
<td>20</td>
<td>37</td>
<td>9</td>
<td>44</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grasshoppers,</td>
<td>Orthoptera</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crickets</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moths</td>
<td>Lepidoptera</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>True Bugs</td>
<td>Hemiptera</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>7</td>
<td>4</td>
<td>6</td>
<td>3</td>
<td>4</td>
<td>6</td>
<td>5</td>
<td>8</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Chapter 3: Pollinator assemblages

**Insect community composition response to flowering season and site type**

Distinct insect assemblages were observed within all study sites between the flowering seasons of the two *Banksia* species. The NMDS plot of the insects collected during *B. attenuata* and *B. menziesii* flowering reached a convergent two-dimensional solution with a ‘fair’ stress of 17% (Figure 3.3A). The ordination illustrates structural dissimilarity between species of insects within sites being grouped apart by flowering season. ANOSIM models for each flowering season showed significant differences between the insect assemblages (Global R=0.34, P=0.001).

Assemblages of the most dominant orders, Hymenoptera, Lepidoptera, Hemiptera and Coleoptera, were not significantly different between natural and restored sites (ANOSIM always Global R < 0.25 and P > 0.05). As differences in insect assemblages within flowering seasons (summer and winter) were found (Fig 3.3), insect functional groups were combined to test similarities between sites. The NMDS plot of sites based on insect functional groups reached convergent two-dimensional solutions with a stress of 8% (good) (Fig. 3.4). The ordination illustrates structural dissimilarity among sites clearly displaying three main groups sharing 75% similarity. Fragmented sites (MC, PP, and AP) comprise one group, while the fourth fragmented site HP was grouped with the continuous sites (NS and NN) and the nearby Gnangara sites (GN and GR). The southern sites at Jandakot (JN and JR) formed a third group (Fig. 3.4, ANOSIM Global R = 0.54, P = 0.002). Insects belonging to the three functional groups, nectar pollen feeders, parasitic, and generalists, contributed to the greatest percentage difference between sites (> 15%; Fig. 3.4).

The SIMPER analysis showed that only four insect species contributed to more than 5% of the differences in any of the taxa. A Dipteran species was more common in all but fragmented sites and an unidentified parasitic wasp species (Evaniidae) was least common in the Jandakot sites (JN and JR). Introduced honeybees, *Apis mellifera*, were least common in the continuous sites (NN and NS), and opportunistic species of Formicidae (ant) were more common in restored sites (JR: N = 122 and GR: N = 25; Appendix 1). Nectar-feeding Pompilidae were most abundant in Jandakot restored site (JR: N = 23; Appendix 1).
Figure 3.3 Non-metric multidimensional scaling (NMDS) plots (resemblance by Bray-Curtis similarity) showing clustering of presence and abundance of species in terms of flowering Banksia species, B. attenuata (summer) and B. menziesii (Schweiger et al.). A, displays invertebrates sampled from Malaise Traps and B, pollinating species observed from point counts. AP, Alexander Park; HP, Highview Park; PP, Paloma Park; MC, Marangaroo Conservation Area; NN, Neaves North; NS, Neaves South; GN, Gungara natural; JN, Jandakot natural; GR, Gungara restored and JR, Jandakot restored.
Figure 3.4 Non-metric multidimensional scaling (NMDS) plots (resemblance by Bray-Curtis similarity) showing clustering of invertebrate functional types by site type. Species sampled from Malaise Traps and combined for flowering banksias. Overlaid clusters indicate similarity at levels of 60% (black line) and 75% (blue line). AP, Alexander Park; HP, Highview Park; PP, Paloma Park; MC, Marangaroo Conservation Reserve; NN, Neaves North; NS, Neaves South; GN, Gnangara natural; JN, Jandakot natural; GR, Gnangara restored and JR, Jandakot restored.
Bird assemblages

A total of 1705 bird and 3001 insect pollinator observations of 26 species were recorded during 192 censuses. Seven species of honeyeaters, the Silveryeye (*Zosterops lateralis*) and Rainbow Lorikeet (*Trichoglossus haematodus*) were observed during the three-year study. The six most abundant observed honeyeaters in decreasing order were Brown Honeyeater, White-Cheeked Honeyeater, Western Wattlebird, New Holland Honeyeater, Red Wattlebird and Western Spinebill. (Fig. 3.5). Total pollinator species richness and abundance was not significantly different (*P* = 0.06) between natural continuous, fragmented and restored sites, however there was a trend of lower bird species richness within fragmented sites (Appendix A3.2).

The most common insectivorous birds observed foraging on multiple inflorescences within natural sites were: the Splendid Fairy-wren (*Malurus splendens*), Western Gerygone (*Gerygone fusca*), Rufous Whistler (*Pachycephala rufiventris*), Willy Wagtail (*Rhipidura leucophrys*), Australian Ringneck Parrot (*Barnardius zonarius*) and Rainbow Lorikeet (*Trichoglossus haematodus*). Less commonly observed birds included White-Breasted Robins (*Eopsaltria georgiana*), Scarlet Robins (*Petroica boodang*), and Australian Golden Whistlers (*Pachycephala pectoralis*).
Figure 3.5 Nectarivores birds observed in the *Banksia* woodlands of the Swan Coastal Plain, A: *Anthochaera carunculata* (Red Wattlebird), B: *Anthochaera lunulata* (Western Wattlebird), C: *Trichoglossus haematodus* (Rainbow Lorikeet), D: *Phylidonyris nigra* (White-cheeked Honeyeater), E: *Phylidonyris novaehollandiae* (New Holland Honeyeater), F: *Lichenostomus virescens* (Singing Honeyeater), G: *Acanthorhynchus superciliosus* (Western Spinebill), H: *Lichmera indistinct* (Brown Honeyeater) and *Zosterops lateralis* (Silereye or Wax-eye).
Pollinator community (birds and insect) composition response to flowering season and site type

No distinct pollinator assemblages were displayed between the flowering seasons of the two *Banksia* species. The NMDS plot of observed pollinators during *B. attenuata* and *B. menziesii* flowering illustrates no structural dissimilarity between species of pollinators within sites by flowering season (Fig. 3.3B). ANOSIM models for each flowering season showed no significant differences between the pollinator assemblages (Global $R = 0.04$, $P < 0.05$). However, the composition of pollinator species differed significantly between sites (Fig. 3.6, ANOSIM Global $R = 0.51$, $P = 0.001$). An NMDS plot of pollinator species reached a convergent two-dimensional solution with a ‘fair’ stress of 17%. Sites were distinguished with three different groupings sharing 60% similarity indicating that fragmented sites (AP, PP, HP, MC) support a different pollinator composition to southern Jandakot sites (JN and JR) and continuous sites (NN and NS) with the Gnangara sites (GN and GR) (Fig. 3.6).

No single species was responsible for the observed dissimilarity between natural and restored sites, but rather it was the difference in the abundance of the most dominant honeyeater species (ANOSIM Global $R = 0.67$ and $P = 0.001$). The analysis of similarity (SIMPER) showed that the six most abundant honeyeater species contributed to more than 12% of the difference between sites. Adjacent (GN and JN) and restored (GR and JR) sites were the least dissimilar (sharing <47% species composition), with fragmented sites the most dissimilar to continuous natural and restored sites (sharing < 20% species composition). There was the greatest species dissimilarity among fragmented sites (sharing only 23% similarity), indicating individual site factors driving differences in assemblages more so than shape or degree of isolation.

Pollinating bird and insect species visitation

A total of 1097 individual birds were recorded in foraging activity, 228 within natural continuous sites, 300 within fragmented sites, 345 within adjacent sites, and 224 in restored sites. Foraging activity was higher during the autumn/winter *B. menziesii* flowering season (n = 623) than the summer flowering *B. attenuata* (n = 474). The species most often encountered in foraging activity were White-cheeked Honeyeaters, Brown Honeyeaters, Western Wattlebirds and European Honeybees. A significant
individual species response to restored sites was observed in two species, Red Wattlebird (*Anthochaera carunculata*) and the Western Spinebill (*Acanthorhynchus superciliosus*). No Western Spinebills were recorded foraging in either restored site (Figure 3.7). The foraging activity of Western Wattlebird within restored sites was more frequent, while foraging of smaller bird species was less frequent (Fig. 3.7). European Honeybee inflorescence visitation was greater ($F = 7.44$, df = 3, $P < 0.001$) in fragmented and restored sites than continuous sites (Fig. 3.8). Native bee visitation was much lower than introduced honeybees across all sites, particularly in Gnangara restored and in Jandakot restored (Fig. 3.8).

Continuous natural sites had overall less honeybee visitation and higher native bee foraging than other site types. The greatest honeybee visitation occurred within the fragmented sites during the summer flowering season of *B. attenuata* (Fig. 3.8). Honeybees visited inflorescences more consistently throughout the day in comparison to honeyeater visitation. Honeybee visitation was comparable to honeyeater visitation during the autumn/winter flowering of *B. menziesii*, with the greatest honeyeater visitation occurring within restored sites in the winter (Figure 3.8). Honeyeater visitation within all sites tended to peak in the morning hours (0600-0900) and decrease throughout the day (Fig. 3.8).
Figure 3.6 Non-metric multidimensional scaling (NMDS) plots (resemblance by Bray-Curtis similarity), showing clustering of presence and abundance of pollinators (bird and invertebrate) from observed point counts for both flowering species *Banksia attenuata* (summer) and *B. menziesii* (winter), produced from NMDS using Bray-Curtis dissimilarity measure with overlaid clusters at a similarity level of 60% (black line) and 75% (blue line).

AP, Alexander Park; HP, Highview Park; PP, Paloma Park; MC, Marangaroo Conservation Area; NN, Neaves North; NS, Neaves South; GN, Gnangara natural; JN, Jandakot natural; GR, Gnangara restored and JR, Jandakot restored.
Figure 3.7 Banksia woodland sites: Non-metric multidimensional scaling (NMDS) of pollinator abundance between sites with superimposed ‘bubble’ plots indicating observed visitation of; Red Wattlebirds, Western Wattlebirds, New Holland Honeyeaters, Brown Honeyeaters, Western Spinebills, White-cheeked Honeyeaters, European Honeybees, and Native bees. Circle size corresponds to the number of foraging counts surveyed within each site and colour corresponds to site type.
Figure 3.8 Differences in pollinator visitation among site type and flowering season: summer flowering *Banksia attenuata* (top) and autumn/winter flowering *Banksia menziesii* (bottom). Mean (± SE) numbers of visits were recorded per 10-minute survey every hour for birds, native insects, and invasive introduced European Honeybees. Visits were recorded for 10 minutes every hour within 30 minutes after sunrise with four surveys each flowering season, at each site, over 3 years (2010-2013).
Foraging behavior of pollinating bird and insect species

Intra-tree movements were uniform across all sites, demonstrating the general foraging movements of honeyeaters (Fig. 3.9 and 3.10). Near-neighbour movements were more frequent within restored sites, with significantly lower frequency of distant tree movements within restored sites ($F = 4.75, df = 3 P < 0.001$). The NMDS plot of all sites grouped both continuous sites (NS and NN) and both natural adjacent (GN and JN) sites together (Fig. 3.8). Honeyeaters travelling out of sites after foraging occurred less frequently in two of the four fragmented sites (HP and PP), likely due to the observation of nesting sites within them (AR, personal observation). Overall, a greater proportion of observed movements to distant trees were recorded for Brown Honeyeaters, White-cheeked Honeyeaters and New Holland Honeyeaters, due to their displacement by larger honeyeaters (Fig. 3.11). Aggressive chases and displacement of foraging honeyeaters was observed at all sites, except Paloma Park (Fig. 3.11). The largest amount of interaction between multiple species was observed within natural continuous and adjacent sites. Intra-species and inter-species aggression interrupted foraging, and chases by larger bodied species were higher in frequency within restored sites compared to the others (Gnangara restored $= 28$, Jandakot restored $= 20$) (Fig. 3.11).
Figure 3.9 The proportions of intra-tree (I), near neighbor (N), distant (D), to *Adenanthos* (shrub species) (A) and those that flew out of the site (F) movements by honeyeaters between foraging bouts on *Banksia attenuata* and *B. menziesii* inflorescences within fragmented, continuous and restored sites.

Figure 3.10 Non-metric multidimensional scaling (NMDS) ordination of pollinator movements during and after foraging bouts on *Banksia attenuata* and *B. menziesii* inflorescences within fragmented, continuous and restored sites AP, Alexander Park, HP, Highview Park, PP, Paloma Park, MC, Marangaroo Conservation Reserve, NN, Neaves North, NS, Neaves South, GN, Gnangara natural, JN, Jandakot natural, GR, Gnangara restored and JR, Jandakot restored.
Figure 3.11 Aggressive chases by birds recorded in all study sites and Banksia species flowering seasons. Numbers within circles indicate intraspecific chases and arrows indicate direction of chases. Circle size indicates proportional body weight (g), sourced from Ford (1979), Newland and Wooller (1985) and McFarland (1986). Bird names in bold text are nectarivores.
DISCUSSION

Understanding the temporal and spatial variability of insect, bird and plant-pollinator mutualisms within natural sites is critical to evaluate their re-establishment within restored sites (Burkle & Alarcon, 2011). Insect assemblages within Banksia woodlands were found to differ between flowering season of *B. attenuata* (summer) and *B. menziesii* (autumn/winter), irrespective of site type. The composition of insect functional groups varied between site type and location. The trend in the data was that both restored sites displayed generally greater species richness, diversity and effective number of species than their natural adjacent sites. Insect species richness, abundance and diversity measures were highest within natural continuous sites and poorest within the smallest fragmented, and one pair of restored and adjacent natural sites. Observed insect and bird pollinator communities did not differ between seasons, however, there were differences between site types. (Small disturbed) fragmented sites contained lower bird species richness than natural continuous sites, a trend observed in many other studies (Ford et al., 2001; Marzluff, 2001; Luck & Daily, 2003; Ford et al., 2009) Restored sites contained similar pollinator species composition to natural sites, although they differed in bird species visitation, foraging movements and behaviour. The pattern of movement among plants also differed between restored and natural sites, with restored sites having higher intra-tree, near-neighbour movement and aggressive chases. Insect and honeybee visitation was greater in summer flowering *B. attenuata* than winter flowering *B. menziesii*, and greater within fragmented sites compared to continuous sites. Honeyeater visitation was greater within restored *B. menziesii* sites, likely due to higher *B. menziesii* inflorescence production. This informs restoration efforts by identifying how habitat composition and structure influences pollinator activity (abundance, visitation and behaviour), which may be crucial to reproductive success in these long-lived species, and the ultimate success of ecological restoration projects.

In the current study, small, but significant, seasonal differences were found in the insect assemblages of the summer flowering *B. attenuata* and the autumn/winter flowering *B. menziesii*. Seasonal differences in insect communities are not unexpected considering climate is one of the factors controlling population fluctuations in ectothermic species (Frazier et al., 2006; Yamamura et al., 2006; Deutsch et al., 2008). Restoration projects
need to consider the restoration of an ecosystem as dynamic, not static, state, as the influence of habitat elements at a variety of spatial and temporal scales includes processes that will maintain the restored site over time (George & Zack, 2001; Tylianakis et al., 2005). Information on the distribution and abundance of species within natural (or reference) sites can be used to identify the habitat elements necessary for target and non-target animal species.

Both restored sites had high abundance of ants (Formicidae), particularly in the Jandakot site, which also contained a higher abundance of nectar-feeding Pompilid wasps than any other site or site type. Formicidae contain generalist species that colonized newly restored sites (Lomov et al., 2009) whilst more specialist species remain within remnant sites (Majer et al., 2007). Pompilid wasps are noted to inhabit sandy areas suitable for nest building (Gwynne, 1979), and the large areas of bare sand suitable for nest building for these solitary wasps and at the Jandakot restored site could explain their higher abundance. The differences in insect functional group composition across fragmented, continuous and restored sites can change at a habitat scale, for example in response to temperature, food availability and provisions for nesting (Lancaster & Rees, 1979; Marzluff & Ewing, 2001; Tews et al., 2004). Reporting species richness alone is an inappropriate measure of diversity to evaluate these restored sites and closer examination of functional groups may reveal more clearly the processes influencing habitat selection and colonization (Gibb & Hochuli, 2002; Majer et al., 2007).

The greater visitation of European Honeybees within fragmented sites (Fig. 3.6) compared to natural continuous sites can be explained by their broad diet, longer foraging ranges and the ability to locate and utilise isolated patches of resources in the wider landscape than solitary natured native bees (Steffan-Dewenter et al., 2002; Celebrezze & Paton, 2004; Steffan-Dewenter & Westphal, 2008). Several other studies have described this pattern of compositional change with increased abundance of *Apis mellifera* sites and decreased native Hymenoptera within fragmented sites (Aizen & Feinsinger, 2003; Paini, 2004; Brosi et al., 2007; Brosi et al., 2008; Winfree et al., 2011). Honeybees can outcompete many native pollinators, cause local extinctions of native bee species and increase in abundance with decreasing habitat size (Aizen & Feinsinger, 1994).
Twenty-years on and *A. mellifera* is still listed as a Key Threatening process in Australian legislation and is an increasing threat to native pollinators worldwide (see review by Goulson, 2003 and Paini, 2004). If this visitation becomes more prominent than honeyeater species, European Honeybees may become a problematic species if they are ineffective pollinators (Sugden & Pyke, 1991; Paton, 1993) and cannot facilitate pollination in restored and fragmented sites. As Floret opening of *B. menziesii* is known to respond to honeyeaters, and not *A. mellifera* (Ramsey, 1988a) increased abundances of honeybees may be a concern. Pollen limitation (Paton, 1993; Vaughton, 1996; Heliyanto et al., 2005) as well as honeybee efficiency (Whelan & Burbidge, 1980; Paton & Turner, 1985; Vaughton, 1992; Dalgleish, 1999; Wooller & Wooller, 2002) has been documented in Proteaceae. In addition, although recognised as a non-pollinating animal, but a substantial pollen taker, Staphylinid beetles have previously been observed to visit *B. menziesii* inflorescences (Gottsberger, 1985; Ramsey, 1988b), however, none were observed or collected during this study. Australian native pollinators such as jewel beetles (Buprestidae) (Kearns et al., 1998) and native bees *Hylaeus alcyoneus* (Paini & Roberts, 2005) and *Amphylaeus morosus* (Spessa, 1999), have declined with *A. mellifera* invasion and the consequences of these honeybees occurring in higher abundances in fragmented and restored sites may reduce native insect colonization within these sites. How this decrease or loss of invertebrate species effects ecological functions, such as pollination patterns warrants further study.

Bird visitation was lower during the summer flowering of *B. attenuata* than during winter flowering *B. menziesii* in restored sites. Within the Jandakot restored site, *B. attenuata* trees produced significantly less inflorescences than the trees in the adjacent natural site. In contrast, *B. menziesii* trees within the Jandakot restored site produced significantly more inflorescences than the trees within the adjacent natural site. Floral architecture of natural and restored systems is one of the major determining factors of honeyeater attraction and presence as honeyeaters move through the landscape in response to flowering resources (Keast, 1968). Whelan and Burbidge (1980) found that over half of the honeyeaters studied within a reserve on the Swan Coastal Plain carried exclusively non-*Banksia* pollen in summer, most likely due to the greater abundance of co-flowering plant species within *Banksia* woodlands during these months. This finding suggests that the lower honeyeater visitation during summer in these restored sites, particularly in Jandakot, may be attributed to the sites low vegetation complexity containing few native co-flowering species.
Banksia menziesii and B. attenuata are serviced by a suite of at least six common honeyeater species that visit their inflorescences, although the relative pollination effectiveness of the honeyeaters remains unclear. The composition (species and numbers) of honeyeaters varied significantly among site type (continuous, fragmented, adjacent, restored). All six avian pollinator species were present in the continuous natural habitat. However, bird pollinator richness declined in fragmented and restored sites. The absence of foraging Western Spinebills (*Acanthorhynchus superciliosus*) at both restored sites suggests the species is sensitive to disturbance. A comparable study by Comer and Wooller (2002) also noted the absence of Western Spinebills within restored Banksia woodland and suggested that it was not floral architecture that prevented visitation, but possibly aggression by larger honeyeaters.

Smaller honeyeaters were observed spending little to no time on inflorescences due to aggressive chases and interruptions by larger honeyeaters during foraging. This behaviour was predominantly recorded in restored sites, however these interspecific interactions have been reported within many natural sites (Paton & Ford, 1977; Ford & Paton, 1982; Newland & Wooller, 1985; McFarland, 1986; Ramsey, 1988b; Ramsay, 1989; Armstrong, 1991a; Paton, 2000; Phillips et al., 2014). Larger bodied Western Wattlebirds with aggressive New Holland Honeyeaters set up territories within restored sites, dominating the higher nectar resources forcing smaller birds (Western Spinebills and Brown Honeyeaters) out of the sites. Newland and Wooller (1985) noted that these two aggressive species flocked in to dominate natural sites of Banksia woodland when flowering was high, whereas in contrast, smaller honeyeaters were territorial residents, exploiting dispersed flowers throughout the year. Similarly other nectivorous species display aggression with changes in flower density and/or nectar availability such as within New Zealand honeyeaters (Craig & Douglas, 1986) and North American hummingbirds (Kodric-Brown & Brown, 1978; Hixon et al., 1983; Carpenter, 1987). Mitchell and Paton (1990) describe that sugar intake rate increases with body size within honeyeater species and that larger bodied Wattlebirds (Red Wattlebirds) achieve a higher intake of nectar than New Holland Honeyeaters and both achieve higher intake rates than smaller bodied Eastern Spinebills. This finding is likely the reason behind this aggression and dominance hierarchy (Armstrong, 1991a; Mac Nally et al., 2005).
In this study, the behaviour of these honeyeaters reduced distant tree movements and increased near neighbour foraging movements, potentially influencing pollen dispersal distances. Phillips et al. (2014) found no reduction in fruit set or seed germination in sites of *Anigozanthos flavidus* (Haemodoraceae) as differences in foraging bouts and aggression between New Holland Honeyeaters and Western Spinebills may mitigate the potential effects of inbreeding by increasing pollen dispersal distances. Within the current study, adjacent *Banksia* trees within restored sites are unlikely to be more genetically similar than distant trees due to mining practices of establishing sites with the application of stripped topsoil (containing soil seedbank) a mixture of broadcast seed, and planting of tubestock, all of which likely breaks down an significant spatial structure between trees (Ritchie & Krauss, 2012). Therefore the proportion of smaller foraging movements between these neighbouring trees is not expected to result in pollen limitation and inbreeding. The immediate issues of decreased pollen movement to distant plants within restored sites may not be a concern because *Banksia* species are predominantly outcrossed, long-lived and carry a high genetic load (Scott, 1980; Wiens et al., 1987). However, for long-term survival and sustainability of restored ecosystems, restoration practitioners and ecologists need to consider how they manipulate pollinator foraging behaviour through vegetation in project planning (Comer & Wooller, 2002).

Animal behaviour, their use of resources and vegetation within restoration has received little attention (Keddy & Drummond, 1996; Lindell, 2008; Morrison et al., 2010; Shuey, 2013), with few studies directly informing how restoration designs can influence behaviour. For example, Fink et al. (2009) demonstrated that canopy cover, tree species and patch size within restored sites influenced bird visitation. Smith-Ramirez and Armesto (2003) demonstrated that territorial bird pollinators of a Proteaceae species (*Embothrium coccineum*) in Chile were largely restricted to defending clumps of 3-5 adjacent flowering trees, with more birds visiting undefended pasture trees. A study of aggression within pollinating insect taxa of *Brassica rapa* (Brassicaceae) revealed that there was no increase in pollination facilitation with increasing aggressive responses and even with decreased per-plant visitation at higher plant densities, seeds per fruit and seeds per flower increased likely only due to non-local pollen deposition.

To encourage and attract the greatest diversity of species (Eckert et al., 2010; Burkle & Alarcon, 2011), practitioners need to identify the key resources needed to establish ‘pollinator-friendly’ environments (Molina-Montenegro et al., 2008). Observations of
smaller-bodied bird foraging in indicate that understorey species such as *Adenananthos* provide nectar as well as protective cover within *Banksia* woodlands (Comer & Wooller, 2002). Habitat attributes can disrupt plant-pollinator interactions, changing pollinator foraging behaviour and flight distances (Aizen & Feinsinger, 1994; Quesada et al., 2003; Garcia-Robledo, 2010; Devoto et al., 2012). The restoration process can alter the amount of light, water, and nutrients received by plants, which in turn influence the number and size of inflorescences or the amount of nectar or quality of pollen produced (Ceccon et al., 2014). The design of restoration projects may facilitate or impede the recovery of the ecosystem through these effects on animal behaviour (Lindell, 2008).

Restored and adjacent natural sites had the greatest level of similarity (75%) of invertebrate functional types and had similar levels of bird abundance, indicating that adjacent sites aid pollinator migration into restored sites. Connectivity to natural sites (continuous or fragmented) increases the ability for re-colonization of invertebrates and birds into restored sites (Kohler et al., 2008). Both restored sites however, exhibited 15% greater insect species richness than their adjacent natural sites, although the Jandakot restored site had lower insect species richness and diversity in comparison to the Gnangara restored site. Ecological restoration of degraded sites is likely to be more functionally successful when these sites are adjacent to high-quality habitats because pollinator diversity and abundance is enhanced through trans-boundary services (Parsons et al., 2003; Williams, 2011). Particularly with invertebrate pollination, the distance between plant populations and high quality habitats effects their pollination and reproductive success (Steffan-Dewenter & Tscharntke, 1999; Albrecht et al., 2007; Kohler et al., 2008). It is thought that the greater capture of insect species within these restored sites is due to site characteristics i.e. the lack of canopy and sparse understorey, increasing understorey species flowering and attraction, and the ease of entering and moving through the restored system from the adjacent natural sites (see Tomlinson et al., 2014 for ecological energetics).

There are similarities in how site structure within restored sites has influenced species richness in this study and those of Clergeau et al. (2001), that demonstrated bird composition in urban areas was independent of bird diversity within adjacent sites, and that fine-scale site-specific attributes such as density and diversity of vegetation influenced species richness. In addition, Munro et al. (2011) reported that bird species
richness within restored and natural sites was linked to the structural complexity of a site and not floristic richness, with natural vegetation containing unique bird assemblages.

Processes influencing animal species site selection differed across temporal scales. For insects, ecological energetics (physical response to their environment) can be a major influencing factor (Tomlinson et al., 2014), for honeyeaters it is availability of floral and nectar resources and there is a common correlation between temporal and spatial scales, indicating that practitioners need to develop strategies to identify and implement these elements (George & Zack, 2001; Shuey, 2013). As honeyeaters are highly mobile generalist species (being both nectarivorous and insectivorous (Recher & Abbott, 1970; Ford & Paton, 1976) they habitually service many plant species in a natural ecosystem. These indirect interactions among visited plants can be competitive, but also beneficial as they can jointly attract and facilitate pollination to species that may not be able to be maintained in isolation (Callaway, 1995; Bruno et al., 2003; Moeller, 2004). Within restored Banksia woodlands, establishing species that provide a more continuous nectar resource needs consideration. This may extend to bridging species (plants that provide resources over resource-limited times) such as B. menziesii, or magnet species (plants with attractive flowers associated with species with unattractive or small flowers) (Menz et al., 2011) such as Calothamnus (Myrtaceae).

Few studies have demonstrated that the loss of pollinators result in pollen limitation (Larson & Barrett, 1999; Knight et al., 2005), although recent studies from New Zealand (Anderson et al., 2011; Pattemore & Anderson, 2013) demonstrated that the functional extinction of bird pollinators reduced pollination, seed production and plant density in native shrubs. Although non-native species can successfully fill the pollinator niche when the native species become extinct (Cox, 1983), there are many examples where the introduction of the non-native Apis mellifera has been detrimental to plant reproduction as they are inefficient or ineffective pollinators for many Australian native plant species (Paton, 1993; Paton, 2000; England et al., 2001; Celebrezze & Paton, 2004).

Other ecosystem services provided by animal species such as herbivory, decomposers, and seed dispersal, are critical for overall community functionality within restored sites. In addressing this, fewer insectivorous bird species were observed in restored sites
compared to natural sites. There was no evidence that these sites provided a corridor for highly mobile insectivorous and granivorous species such as Rufous-Whistler, Willy Wagtails and robins, which were absent from restored sites. These species require more complex vegetation structure for visitation, establishment and long-term survival. The lack of vegetation complexity in restored sites prevents the development of foraging niches (Barbaro et al., 2012), as a hierarchy of trophic levels fail to form. Plant establishment may eventually lead to succession and these services may restore with time (Marzluff, 2001).

The Society for Ecological Restoration states that an attribute of a restored ecosystem is that it “functions normally” (SER 2004). To create a restored ecosystem that “functions normally”, practitioners need to identify the vegetation components and community structure that will facilitate species visitation across temporal scales (George & Zack, 2001). Although many banksias are buffered against the loss of some pollinators by having generalised pollination systems including birds and insects (Whelan & Burbidge, 1980; Coates et al., 2007), determining the level to which functionality is restored within restored plant communities is challenging. Pollinators contribute to ecological functions that are critical to the long-term stability of ecosystems, but the exact identities of these species may be less important than having a diverse mixture of functional groups (Klein et al., 2008). More broadly, if these pollinator assemblages are facilitating comparable levels of functionality to that of historic or natural reference sites, the restoration goal of ecosystem function and stability may have been achieved (Kremen, 2005).

The spatial variability of insect and bird assemblages within Banksia woodlands in an urban landscape matrix revealed that the choice of a ‘reference’ site for restoration must encompass different site types. Given the response of functional groups to restoration, assessments of restoration programs in urban bushland fragments should focus on functional characteristics of faunal responses, rather than simple taxonomic measures. In order to evaluate restoration success, simply measuring species richness and diversity is not enough to create resilient self-sustaining ecosystems (White & Walker, 1997). Restoration projects should aim to revegetate areas in a manner that maximises the return of a full range of biodiversity (Majer et al., 2007) across different functional groups. The restoration of self-sustaining sites and key population processes without the future aid of human management, requires the establishment of plant species rich
communities and more importantly vegetation structure which takes time (Dobson et al., 1997; Suding, 2011). A better understanding and evaluation of ecological interactions, such as these complex plant-pollinator relationships, would help in unprecedented ways the practical goals of restoring functionality. Given the response of honeyeater aggression and limited foraging movements in restored sites, costs and benefits of territoriality need investigation, as gene flow through pollen movement is influenced by pollinator behaviour. This information can inform restoration efforts by identifying how the structure and composition of restored vegetation influences behaviour of species that are critical to the survival and reproductive success of the restored site (Lindell, 2008; Fink et al., 2009; Morrison et al., 2010). Dispersed planting of an array of nectar producing species that have overlapping flowering phenologies throughout the year, may encourage more smaller bodied honeyeaters into restored sites (Comer & Wooller, 2002). Achieving these goals may depend on selecting a species set for restoration or in different quantities that may not initially reflect particular reference sites or historical compositions (Shackelford et al., 2013).
Chapter 4: A genetic assessment of reproductive functionality and connectivity in restored populations of *Banksia menziesii*

**ABSTRACT**

The success of ecological restoration relies on the establishment of viable populations that survive to reproduce viable offspring, persisting over generations. Restoring functional ecosystem services such as pollinator communities are critical to ensure the successful maintenance of plant reproduction. To assess genetic aspects of reproductive functionality, reproductive output, mating system parameters, paternity and direct and indirect measures of pollen movement were estimated for restored and natural sites. Microsatellite DNA markers were used to genotype adults and seed for a low and high complexity restoration site and an array of natural sites of *Banksia menziesii*. Genotypic data were analysed using direct measures of paternity assignment (CERVUS) and indirect methods of mating system characterisation (MLTR) and pollen dispersal (TwoGener and Pollination Graphs). To assess the relationship between pollinator visitation and reproductive output, follicle and seed production per inflorescence were surveyed within restored and natural sites. Paternity assignments revealed effective pollen dispersal that departed from predominantly nearest neighbour mating, in contrast to observed pollinator foraging movements. Realised pollen dispersal distances were extensive within and among sites, with the exception of the low complexity restored site. Pollen networks showed an association between reduced pollinator services and low genetic connectivity in the low complexity restored site. The lowest differentiation in pollen pools was within the low complexity restored site ($\Phi_{st} = 0.043$) and the highest in the complexity site ($\Phi_{st} = 0.081$), but overall all sites displayed high levels of differentiation among families ($\Phi_{st}$ range = 0.043-0.087) indicating multiple pollen donors per family. Mating system analysis showed *B. menziesii* was effectively outcrossed in all sites ($t_m > 1.0$) with low estimates of bi-parental inbreeding ($t_m - t_i = -0.027 – 0.231$) and correlated paternity ($r_p = 0.061 – 0.159$). The highest estimates of effective pollen donors per maternal ($N_{ep}$) were recorded in the low complexity restored site (MLTR; $N_{ep} = 16.4$) and the continuous natural site (MLTR; $N_{ep} = 12.5$). Follicle to
inflorescence ratio was not significantly different among sites (mean = 4.47 follicles per inflorescence) and although the low complexity restored site had significantly higher follicle production per tree (140.3, range = 61.1 – 219.5 follicles per natural tree), the average percentage of viable seed produced was the lowest (68.5%, range = 56.2 – 80.8%). Results suggest that reduced potential pollen dispersal within the low complexity restored site has reduced realised dispersal despite the high levels of pollen donors, has lower genetic differentiation among families, lower connectivity to its adjacent natural site and less viable seed produced.

INTRODUCTION

The pollination of flowering plants is essential to ecosystem function (Aizen & Feinsinger, 2003; Ghazoul, 2005; Kremen et al., 2007b; Lonsdorf et al., 2009; Menz et al., 2011), with 87% of terrestrial angiosperms requiring pollinators (Friedman & Barrett, 2009; Ollerton et al., 2011). Foraging behaviour of pollinators influence the mating system, number of potential mates and ultimately the reproductive success of plants (Wilson & Thomson, 1991; Dick et al., 2003; Rymer et al., 2005; Rymer et al., 2010). Pollinators are largely influenced by the landscape features such as the density of flowering plants, distribution and size of the site as well as the composition of pollinator communities (Kearns & Inouye, 1997; Byrne et al., 2007; Steffan-Dewenter & Westphal, 2008). In anthropogenic-disturbed landscapes, habitat degradation, fragmentation and loss can cause decline in plant and/or pollinator populations, potentially leading to pollination limitation (Knight et al., 2005; Aguilar et al., 2006; Kremen et al., 2007a; Winfree et al., 2011).

Globally, the major threatening processes to natural ecosystems are anthropogenic through changes in land use and habitat degradation (Dobson et al., 1997). A key strategy to address these damaging processes is to implement restoration programs (Hobbs & Cramer, 2008; García-Robledo, 2010). A key goal of ecological restoration is to re-establish self-sustaining ecosystems, with the ability to persist in dynamic settings in the short-term and the capacity to undergo adaptive evolutionary change in the long term (SER, 2004). Restoring plant-pollinator processes are vital for restored population success, yet has received little attention (Dixon, 2009; Menz et al., 2011). An understanding of species pollination systems, gene flow, seed production and patterns of
Chapter 4: Pollen movement

pollinator foraging behaviour (Rymer et al., 2010; Garibaldi et al., 2011) is therefore required. In restored sites, shifts in pollinator abundance diversity and behaviour can result in pollen limitation, reduced seed set and lower outcrossing (Kearns & Inouye, 1997; Montalvo et al., 1997; García-Robledo, 2010). Due to their effect on plant reproduction and gene flow, the failure to manage and promote pollinators could lead to decline or failure of ecological restoration efforts.

Plants within fragmented landscapes have a greater risk of pollination failure with reduced pollinator visitation and or increased self-pollen deposition (Wilcock & Neiland, 2002), therefore have been the focus of many studies (Aizen & Feinsinger, 1994; Kevan et al., 1997; Ghazoul & McLeish, 2001) however, there are few studies in a restoration context. The restoration of inter-specific interactions such as plant-pollinator relations is less understood (Forup & Memmott, 2005; García-Robledo, 2010; Burkle & Alarcon, 2011; Devoto et al., 2012). Previous findings have shown that as habitat fragmentation intensifies with increased land use, pollinator diversity, abundance and pollinator services are affected (Steffan-Dewenter & Westphal, 2008). Fragments may be left without the services of reliable pollinators and suffer diminished fruit set (Aizen & Feinsinger, 1994; Aguirre & Dirzo, 2008; Brosi et al., 2008; Klank et al., 2010), reduced outcrossing (Sipes & Tepedino, 1995 although see Dick, 2001; White et al., 2002) and low genetic diversity (Menges, 1991; Debinski & Holt, 2000), reviewed in Oostermeijer et al., 2003).

Other studies show that fragmentation can result in increased long-distance gene flow through pollen dispersal (Dick et al., 2003; Byrne et al., 2007) and observed to be buffered against the predicted negative population genetic effects of fragmentation (Lowe et al., 2005; Kramer et al., 2008; Breed et al., 2012a). Few studies have shown greater long-distance foraging in vertebrate (avi-fauna) versus invertebrate (Hymenoptera) (Heinrich, 1975; Brown et al., 1978; Stiles, 1978), with avian-pollinated plants having lower population genetic divergence than invertebrate-pollinated species (Byrne et al., 2007; Krauss et al., 2009; Kramer et al., 2011). Visitation by birds to more flowers or inflorescences on different plants may increase pollination, in terms of both the quantity and quality of pollen deposited (McDade & Kinsman, 1980) and the genetic quality of progeny (England et al., 2001). However, if nectar resources are at levels that are insufficient to sustain pollinators, a decrease in reproductive success may
follow (Garibaldi et al., 2011; Cranmer et al., 2012). How these pollination systems are influenced by or created within restored sites is poorly understood (García-Robledo, 2010; Kaiser-Bunbury et al., 2010; Winfree, 2010; Menz et al., 2011; Williams, 2011).

Molecular markers can be used to determine realised pollen movements, and therefore the effective movement of pollinators at small and large scales (England et al., 2001; Krauss et al., 2009). For example, using a paternity assignment approach, plants in a population of *Calothamnus* were pollinated by avifauna (honeyeaters; Meliphagidae) travelling between fragments over 5 km away (Byrne et al., 2007), when more local scale studies using observational data found they move on average 4 m between plants (Hopper & Moran 1981). Assigning paternity in a population of *Banksia hookeriana* (Krauss et al., 2009) and in populations of *Banksia attenuata* (Ritchie & Krauss, 2012), realised pollen dispersal was found to depart from typically nearest neighbour mating, with significant pollen immigration. These studies directly inform on the genetic consequences for plants of pollinator abundance, movement and behavior (England et al., 2001).

Indirect analyses of pollen-mediated gene flow based on two-generation (parent-offspring) genetic structure can be used to identify the scale of male gametic heterogeneity among females, allowing the interpretation of the average distance of pollen movement (Smouse et al., 2001). This approach foregoes the need for identification of pollen donors, and categorizes collections of individual pollination events, and allows for study of landscape spatial scale pollen movement en mass (Dyer et al., 2012) and may provide an indication of the pollinator dispersal capabilities within the landscape.

Previous genetic studies in *Banksia* (Krauss et al., 2009; Ritchie & Krauss 2012) show evidence of extensive pollen movements within and among sites due to highly mobile bird pollinators. Bird pollinated species tend to have highly outcrossed mating systems with extensive multiple paternity and near random mating (Wooller & Wooller, 2002). In contrast the foraging behavior of insects such as honeybees have been noted to make smaller infrequent interplant movements (Paton, 1993; Richardson et al., 2000; Whelan et al., 2009), limiting their effectiveness as pollinators to self-incompatible species (Paton, 1993). Through caged experiments of Proteaceae, in which vertebrate pollinators were excluded, pollination by honeybees was revealed, however the quantity
of seed produced was significantly lower than when bird or marsupial pollinators had access to flowers (Vaughton, 1992). The *Banksia* (Proteaceae) genus displays the highest outcrossing rates seen among plants (Scott, 1980; Wooller & Wooller, 2002; Coates et al., 2007; Lamont et al., 2007). Whelan and Burbidge (1980) “feeding activities of honeyeaters might therefore be expected to affect patterns of seed set in these *Banksia* species”. *Banksia menziesii* has mixed generalist pollinators and are reliant on their services for mating. The species warrants study, as pollinator communities and their diversity can change with land degradation (Brosi *et al*. 2008; Steffan-Dewenter & Westphal 2008), it is vital that these plant-pollinator interactions are restored for reproductive function in this species.

In this study I address the following questions by assessing and evaluating the actual distance of pollen dispersal events by assigning parentage to seeds. I then analyse spatial and genetic differentiation of pollen pools and construct pollen dispersal curves. Calculating the percentage of pollen flow entering from outside the studied site then allows the assessment of the importance of adjacent sites as sources of gene flow and assessing if there is a correlation of realised pollen dispersal, reproductive output and the observed frequency and visitation of pollinators within each site.

Specifically, I addressed the following questions:

(i) Is reproductive output of *Banksia menziesii* in restored sites lower than that of natural (continuous and fragmented) sites?

(ii) Does pollinator visitation correlate with reproductive output within restored and natural (continuous and fragmented) sites of *Banksia menziesii* sites?

(iii) Do mating systems parameters, and pollen dispersal networks of *Banksia menziesii* in restored sites differ from natural (continuous and fragmented) sites?
Chapter 4: Pollen movement

METHODS

**Study species; flowering characteristics and pollinators of Banksia menziesii**

*Banksia* is an endemic Australian genus containing over 170 species (Collins et al. 2008; Cardillo and Pratt 2013). *Banksia menziesii* is a widespread tree or woody shrub species, distributed across the Swan Coastal Plain of Western Australia, on deep sand in kwongan, shrubland and woodlands (George 1981; Corrick & Fuhrer 2002). It is a dominant species used extensively in ecological restoration of sand quarries in the region (Rokich et al. 2002).

*Banksia menziesii* has about 600-1400 pink to red (some yellow) sessile florets (37-71mm long) and are arranged orthogonally around a central woody axis, forming inflorescences 4-12cm long (George 1981; Ramsey 1986). Prior to floret opening, pollen is deposited from sessile anthers onto a modified pollen presenter at the style-end, around the terminal stigmatic groove. After floret opening the style protrudes beyond the relaxed perianth (George 1981). Floret opening on inflorescences proceeds acropetally, with 30-70 opening per 24 hours (Ramsey 1988). Of these, approximately 95% open during daytime, mostly in response to foraging by nectarivorous birds. By dusk, all of the pollen has been removed from florets suggesting nocturnal pollination is unlikely (Ramsey 1986, 1987). The species flowers during autumn/winter (April-August) and is most likely the major source of floral nectar for honeyeaters in the winter months (Ramsay, 1989).

*Banksia menziesii* provides an important source of nectar for a wide range of nectar-feeding birds including the Brown Honeyeater (*Lichmera indistincta*), White-cheeked Honeyeater (*Phylidonyris nigra*), New Holland Honeyeater (*Phylidonyris novaehollandiae*), Singing Honeyeater (*Lichenostomus virescens*), Red Wattlebird (*Anthochaera carunculata*), Western Wattlebird (*Anthochaera lunulata*), and Western Spinebill (*Acanthorhynchus superciliosus*) (Whelan & Burbidge, 1980; Ramsay, 1989; Chapter 3) and, potentially, small mammals such as the Honey Possum (Hopper 1980). The woody infructescences are serotinous, viable seeds being retained within the follicles for a short period of time and only released following fire or heat (Lamont et al., 2007)
Study sites

This genetic study was conducted in five natural and two restored *Banksia* woodland sites on the Swan Coastal Plain, within the southwest region of Western Australia. A range of *Banksia* woodland sites were selected to assess the reference landscape for a genetic assessment of restoration success. The sites were: one continuous woodland site, two fragmented *Banksia* woodland reserves two woodland sites adjacent to two restored sites. The natural continuous site was located within an area of 5 Hectares chosen from within the Gnangara-Moore River State forest, Neaves North (NN). The two fragmented sites were located within an urban landscape Alexander (now known as Hepburn) Reserve (AP) and Highview Park (HP). The two adjacent woodland sites were neighbouring each of the two restored sites, Jandakot natural (JN) and Gnangara natural (GN). Rocla Quarry Products restored the two sites being assessed, Jandakot Restored site (JR) and Gnangara Restored site (GR). For the assessment of reproductive output, another three natural sites were added to the study, one continuous natural site also residing within the Gnangara-Moore River State forest; Neaves South (NS) and two fragmented sites; Marangaroo Conservation Area (MC) and Paloma Park (PP). Chapter 2 contains the full site descriptions and maps.

Assessment of reproductive output

To measure reproductive output, ten adult trees per site were selected randomly and tagged in 2010. For each tree multiple counts of new inflorescences produced were recorded and the number of cones that bore follicles (infructescence) in each of three years (2010-2012). In the first year of the study (2010), each infructescence was collected from each selected tree. To extract the seeds, each cone was placed in a hessian bag and soaked for 1 hour in water, then placed in an oven set at 150°C for 10-15 minutes until the follicle rupture and seeds could be removed. Collected cones were assessed for number of follicles, fertile seeds, evidence of seed predation, infertile and aborted embryos (non-viable), to give a count of seed per cone per plant. Seeds were weighed and average weight for each plant estimated. A selection of ten viable seeds from each family was later used for genetic studies. For two years following (2011-2012), reproductive output was measured by counting inflorescences produced, the number of infructescences and the number of follicles produced per infructescence, for each of the selected trees.
Pollinator foraging observations

Two trees with flowering inflorescences and their surrounding neighbours were monitored at each site within each flowering season during the Austral autumn (April to June) over each of three years (2010-2012). Trees were monitored by the same observer (AR) and one volunteer once each season in 2010/2011 and twice each season thereafter, during days without rain or high wind. On each day, visits to inflorescences by invertebrate and avian species were observed for eight, 10 minute census periods starting within 30 minutes of sunrise. To assess the relationship between reproductive output and pollinator visitation I used a simple linear regression model to test for an association between (a) pollinator visitation and follicles produced, inflorescences and inflorescence:follicle ratio, (b) inflorescence production and follicle production, (c) pollinator type (ie. avian or honeybee) visitation and inflorescence: follicle ratio. Visitor frequency to inflorescences was calculated by dividing the total number of visits recorded in a 10 minute observation period by the number of inflorescences being observed for this time. This measure was averaged over all the 10 minute observation periods and converted to the number of visits per tree per day per site.

Genetic assessment

Genetic assessments of mating, paternity and pollen flow were conducted for both restored sites (Jandakot restored (JR) and Gnangara restored (GR)) and their adjacent remnants (Jandakot natural (JN) and Gnangara natural (GN)), as well as fragmented Banksia woodland sites, Alexander Park (AP) and Highview Park (HP) and the continuous natural site Neaves North (NN).

Leaf samples were collected from mature Banksia menziesii trees during December 2010- January 2011. Approximately 100 trees were sampled within each site (JR, GR, JN, GN, AP, HP and NN), within a given area of 200m². Ten trees with the most seed-bearing cones were selected from the same flowering period from which a minimum of 4 cones could be collected, in order to collect at least 10 seeds per plant. Exact locations of all individual trees within an approximate area of 200 m² were recorded by global positioning system (GPS) for density and those genetically sampled for spatial analyses. The number of flowering individuals from the previous year was estimated for the given size of the site by counting trees based on age class of floral remnant persistence and/or infructescence follicle colour (Crawford et al. 2010).
Genomic DNA was extracted from leaves dried in silica gel using a cetyltrimethylammonium bromide (CTAB) method modified from Doyle and Doyle (1987) for Banksia (He et al. 2004). Seed coats were separated from their radicles to avoid contamination of seed DNA by maternal tissue. Radicles were pulverized (directly in the extraction buffer with one glass bead and 0.5 g acid washed sand in a 2 ml tube) within a tissue and cell homogenizer FastPrep®-24 instrument (MP Biomedicals, Inc., Solon, OH). The instrument was set for 24 samples of 2 ml tubes and ran for 20 seconds. DNA pellets were resuspended in 75 µL of 10 mM Tris and 1 mM EDTA, and 2 µL (of 5 µg DNA/100 µL dH2O.) of this mixture was used in each polymerase chain reaction (PCR).

**Characterization of microsatellite markers**

Microsatellite amplifications were performed for eleven polymorphic markers (BmA1, BmB6, BmB102, BmB106, BmC2, BmD1, BmD4, BmD103 and BmD105) previously designed for B. menziesii (He 2013) (Appendix 2) and two (BaA3, BaB106) previously designed for B. attenuata (He et al. 2004; 2007) (Appendix 3). Amplifications were performed with the following PCR conditions: 96°C for 2 min (1 cycle), followed by 30 cycles of 94°C for 1 min, 52 to 57°C for 1 min (according to each primer pair annealing temperature, Appendix 2), 72°C for 1 min and final extension time of 72°C for 7 min. PCR products were stored at 4°C then purified with an isopropanol clean-up (Appendix 2) before being separated by capillary electrophoresis using a Beckman Coulter CEQ 8800 Genetic analysis system. An internal size standard (SS400) was added to each well loaded on the CEQ 8800. Alleles were scored using the CEQ 8800 Genetic Analysis System program.

**Assessment of reproductive output**

To test for variation in inflorescence and follicle production among years and sites, I used repeated measures of Analyses of Variance (ANOVA). Two-factor nested ANOVAs were used to determine whether inflorescence and viable seed production, inflorescence: follicle ratio varied with each site. The two factors in the analyses were landscape type (a fixed factor) and site nested within site size (a random factor). The latter provided a measure of variation among sites within site type categories.
Mating system

Mating system parameters were estimated using MLTR v. 3.4 (Ritland 2002). Maximum-likelihood single \((t_s)\) and multilocus \((t_m)\) estimates of outcrossing rates, biparental inbreeding \((t_s-t_m)\), correlated paternity \((r_p)\) and the effective number of pollen donors per family \((1/r_p)\) was calculated (Smouse et al., 2001). MLTR was used to confirm the inheritance of maternal alleles. The genetic diversity estimates were bootstrapped 100 times by re-sampling loci within families and the 95% confidence interval was estimated (see Ritland 2002).

Paternity assignment

Likelihood-based paternity analysis was conducted using the program CERVUS 2.0 (Marshall et al., 1998; Kalinowski et al., 2007). The likelihood ratio is defined as the likelihood of a paternity of a particular individual relative to the likelihood of paternity of a random individual. Paternity is assigned to a particular individual if the logarithm of combined likelihood ratios derived at each locus, is larger than the likelihood ratios of other individuals. The logarithm of combined likelihood ratio is termed the logarithm of odds (LOD) score. The Delta statistic is defined as the difference in LOD score between the first and the second most-likely candidate parents. Simulations were used to determined the threshold Delta scores at a given confidence level. In this study, the simulation parameters for CERVUS to assign paternity to the most-likely individuals with a known level of statistical confidence were set as follows: 10,000 cycles of simulation, 204 candidate parents (all sampled reproductive adults from both restored and natural populations), 0.01 as the proportion of loci mistyped (genotyping error ratio), confidence levels of 95.0 and 80.0%. To assign an offspring to a pollen donor, a maximum of two mismatches between the offspring, mother tree and putative donor trio was used and the tree with the highest LOD value was accepted as the parent of the seedling if the difference between its LOD score and the second most-likely candidate’s LOD score was greater than the threshold Delta. A maximum pollen dispersal distance (PDD) was calculated from paternal assignments under relaxed confidence (80%) by using GPS location of the maternal and the paternal tree identified as the pollen donor.
Chapter 4: Pollen movement

**Pollen dispersal curve**

The parameters of the pollen dispersal kernels, including the scale (a), shape (b) and average effective pollen dispersal distance (d), were estimated using KINDIST (Robledo-Arnuncio et al. 2006) as implemented with POLDISP (v. 1.0 Robledo-Arnuncio et al. 2007). KINDIST models the decay in a normalized measure of correlated paternity with increasing spatial distance between maternal pairs, and is independent of the density of pollen donors. Correlated paternity was calculated based on pair-wise kinship as the probability of paternal identity between a pair of maternal sibs with geographic distance, z, relative to the average within maternal sibship (Robledo-Arnuncio et al. 2006). This indirect method estimates contemporary pollen dispersal as a function of the genetic structure of pollen pools allowing for unequal male fecundity. The convenience of this approach was validated by testing for a significant decline in correlated paternity with increasing distance using a Spearman's rank correlation. In most populations, there was insufficient points to create a pollen dispersal curve and kernel estimation, therefore this data has been excluded from the results.

**Pollen pool structure**

Differentiation in maternal tree pollen pools ($\Phi_h$) and effective density ($d$) across populations were estimated with TwoGener analysis within GenAlEx 6.5 (Peakall & Smouse, 2012), Effective number of pollen donors per family ($N_{ep}$) was estimated by $1/2 \Phi_h$ and the effective pollination neighbourhood area ($A_{ep}= N_{ep}/d$ (ha)) in TwoGener. The overall molecular differentiation between pollen pools ($\Phi_h$), together with its variance, was calculated following Smouse et al. (2001). The required sampling for TwoGener is less rigorous, as the mean pollination distance and the effective neighbourhood size are estimated from the heterogeneity of alleles within the sampled families and uses plant density estimates (obtained and estimated for trees/ha within each site).

Spatial differentiation of pollen pools was then examined using Pollination Graphs (Dyer et al, 2012), which are based on the Population Graph (PG) approach detailed in Dyer and Nason (2004). PGs analyse pollen pool covariance rather than among-strata adult structure. This graph-theoretic approach describes the spatial distribution of...
genetic covariance among strata using conditional genetic covariance to define a network topology describing multilocus genetic connectivity. In the network that is defined, nodes represent maternally sampled pollen pools, and connecting lines represent links of significant conditional covariance (Dyer et al, 2012). To illustrate the genetic structure of sampled pollen pools, the paternal contribution to each offspring genotype was used. These were inferred from the male pollen multilocus haplotypes for each offspring produced from the Two-Gener analysis. In cases where the maternal individual and offspring had the same heterozygote genotype, the paternal contribution was assigned probabilistically, outlined in the methods of Smouse et al. (2001) (Dyer et al. 2012). The PG was then estimated and visualised in using gstudio and popgraph (Dyer 2009; Dyer et al. 2012) in R statistical package (v 3.1.0, R Core Team 2014). A linear regression analysis was then conducted between Pollen Graph node size, representing the genetic variability of pollen haplotypes sampled by each maternal individual, and inflorescence and follicle production of maternal tree to test for any relationship.

RESULTS

Assessment of reproductive output

Overall, there was a lot of variation in the production of *B. menziesii* inflorescences across sites and years (Fig 4.1a). Inflorescence production among sites was significantly different ($F = 2.83$, df = 9, $P = 0.02$) as well as among landscape type ($F = 3.15$, df = 3, $P = 0.04$). The Jandakot restored site produced the most inflorescences and follicles per maternal tree (140.3, CI= 61.1-219.5) (Table 4.1, Fig 4.1a & b), however, this did not translate into a higher follicle:inflorescence ratio (Fig. 4.1c) and in fact the average percentage of viable seeds produced per maternal was the lowest (68.5%, CI= 56.2-80.8) ($F = 6.98$, df = 9, $P = 0.03$) (Table 4.1). Inflorescence and follicle production at the Gnangara restored site was much more consistent over the three years, and lead to a higher overall follicle:inflorescence ratio than the Jandakot restored site.

There were associations within years (2010 and 2012) between inflorescence and follicle production, the strongest relationship was between number of inflorescences and follicle production in 2010, explaining greater than 26% of the variation in ($P < 0.001$; Fig. 4.2a). There was also a significant association between the number of inflorescences produced and number of visitors (attractiveness of a site to pollinators)
and the number of follicles produced and visitation (successful cross pollination, Fig. 4.2d & e). It is interesting to note that generally higher visitation rates by pollinators in both restored sites lead to higher follicle production (Fig 4.2e), although this did not translate to a higher percentage of viable seeds produced per maternal tree than any of the natural sites (Table 4.1).
Figure 4.1 Mean inflorescence (a), follicle production (b) and follicle to inflorescence ratio (c) per plant in Banksia menziesii sites. Data were collected 2010, 2011 and 2012. Annual rainfall for each site (a) obtained from BOM (www.bom.gov.au). AP, Alexander Park; HP, Highview Park; PP, Paloma Park; MC, Marangaroo Conservation Reserve; NN, Neaves North; NS, Neaves South; GN, Gnangara Natural; JN, Jandakot Natural; GR, Gnangara Restored and JR, Jandakot Restored. Data were not collected at JN and JR in 2010. Standard error bars are shown.
Table 4.1 Reproductive measures of *Banksia menziesii* in continuous, fragmented, adjacent and restored sites. Average number of follicles produced per tree, follicles setting viable seed and the percentage of viable seeds averaged per maternal over all years (and their 95% confidence intervals shown in parentheses).

<table>
<thead>
<tr>
<th>Site</th>
<th>Code</th>
<th>Type</th>
<th>Average no. of follicles produced per maternal tree</th>
<th>Average no. of follicles setting viable seed</th>
<th>Percentage of viable seeds produced per maternal tree</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neaves North</td>
<td>NN</td>
<td>Continuous</td>
<td>46.3 (28.4-64.1)</td>
<td>4.68 (1.81-7.56)</td>
<td>71.1 (53.5-88.6)</td>
</tr>
<tr>
<td>Neaves South</td>
<td>NS</td>
<td>Continuous</td>
<td>25.4 (17.5-33.2)</td>
<td>3.45 (2.37-4.55)</td>
<td>87.8 (76.6-99.1)</td>
</tr>
<tr>
<td>Alexander</td>
<td>AP</td>
<td>Fragmented</td>
<td>34.7 (32.1-37.4)</td>
<td>3.42 (1.41-5.43)</td>
<td>71.4 (61.9-81.0)</td>
</tr>
<tr>
<td>Highview</td>
<td>HP</td>
<td>Fragmented</td>
<td>25.9 (15.0-36.7)</td>
<td>3.88 (2.29-5.48)</td>
<td>80.3 (72.1-88.4)</td>
</tr>
<tr>
<td>Marangaroo</td>
<td>MC</td>
<td>Fragmented</td>
<td>19.4 (16.4-22.4)</td>
<td>5.04 (3.38-7.23)</td>
<td>88.5 (82.7-94.3)</td>
</tr>
<tr>
<td>Paloma</td>
<td>PP</td>
<td>Fragmented</td>
<td>22.1 (13.9-30.2)</td>
<td>5.22 (2.45-7.99)</td>
<td>82.3 (73.2-91.4)</td>
</tr>
<tr>
<td>G nagara Natural</td>
<td>GN</td>
<td>Adjacent</td>
<td>61.7 (28.6-94.8)</td>
<td>4.06 (2.21-5.92)</td>
<td>75.4 (64.8-86.0)</td>
</tr>
<tr>
<td>Jandakot Natural</td>
<td>JN</td>
<td>Adjacent</td>
<td>32.1 (25.6-38.7)</td>
<td>5.75 (4.28-7.22)</td>
<td>76.2 (70.9-81.5)</td>
</tr>
<tr>
<td>G nagara Restored</td>
<td>GR</td>
<td>Restored</td>
<td>49.1 (45.4-52.9)</td>
<td>5.11 (3.73-6.50)</td>
<td>83.5 (67.6-99.4)</td>
</tr>
<tr>
<td>Jandakot Restored</td>
<td>JR</td>
<td>Restored</td>
<td>140.3 (61.1-219.5)</td>
<td>6.96 (4.21-9.70)</td>
<td>68.5 (56.2-80.8)</td>
</tr>
</tbody>
</table>
Figure 4.2 Positive association between (a, b, c) inflorescence number and follicle production for each of three years, (d) pollinator visitation and inflorescence production, and (e) pollinator visitation and follicle production in Banksia menziesii. Five sites were measured in 2010, and 7 sites in 2011 and 2012. Linear regression lines and the results of regression analyses are shown. n = number of plants measured.
Table 4.2 Mating system and pollen gene pool parameters estimated for seven sites of *Banksia menziesii* (95% confidence interval in parentheses).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Continuous Neaves North Natural</th>
<th>Continuous Alexander Natural</th>
<th>Continuous Highview Natural</th>
<th>Adjacent Gnangara Natural</th>
<th>Adjacent Jandakot Natural</th>
<th>Restored Gnangara Restored</th>
<th>Restored Jandakot Restored</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MLTR Mating system</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multilocus outcrossing rate ($t_m$)</td>
<td>1.086</td>
<td>0.984</td>
<td>0.957</td>
<td>1.200</td>
<td>0.986</td>
<td>1.200</td>
<td>1.028</td>
</tr>
<tr>
<td>($0.922-1.250$)</td>
<td>($0.849-1.119$)</td>
<td>($0.840-1.069$)</td>
<td>($1.196-1.203$)</td>
<td>($0.833-1.138$)</td>
<td></td>
<td>($1.196-1.203$)</td>
<td>($0.881-1.175$)</td>
</tr>
<tr>
<td>Singlelocus outcrossing rate ($t_s$)</td>
<td>0.955</td>
<td>0.972</td>
<td>0.941</td>
<td>0.969</td>
<td>1.013</td>
<td>1.015</td>
<td>0.916</td>
</tr>
<tr>
<td>($0.898-1.012$)</td>
<td>($0.927-1.017$)</td>
<td>($0.875-1.008$)</td>
<td>($0.914-1.024$)</td>
<td>($0.944-1.082$)</td>
<td></td>
<td>($0.958-1.0718$)</td>
<td>($0.873-0.959$)</td>
</tr>
<tr>
<td>Biparental inbreeding ($t_m - t_s$)</td>
<td>0.130</td>
<td>0.012</td>
<td>0.016</td>
<td>0.231</td>
<td>-0.027</td>
<td>0.185</td>
<td>0.112</td>
</tr>
<tr>
<td>($-0.05-0.310$)</td>
<td>($-0.117-0.141$)</td>
<td>($0.008-0.024$)</td>
<td>($0.176-0.286$)</td>
<td>($-0.152-0.098$)</td>
<td></td>
<td>($0.128-0.242$)</td>
<td>($-0.023-0.247$)</td>
</tr>
<tr>
<td>Correlated paternity ($r_p$)</td>
<td>0.080</td>
<td>0.153</td>
<td>0.087</td>
<td>0.159</td>
<td>0.135</td>
<td>0.127</td>
<td>0.061</td>
</tr>
<tr>
<td>($0.011-0.149$)</td>
<td>($0.082-0.224$)</td>
<td>($0.003-1.171$)</td>
<td>($0.065-0.253$)</td>
<td>($0.055-0.215$)</td>
<td></td>
<td>($0.045-0.209$)</td>
<td>($0.020-0.102$)</td>
</tr>
<tr>
<td>Effective number of pollen donors $N_{ep} = (1/ r_p)$</td>
<td>12.5</td>
<td>6.5</td>
<td>11.5</td>
<td>6.3</td>
<td>7.4</td>
<td>7.9</td>
<td>16.4</td>
</tr>
<tr>
<td>Effective pollination neighbourhood area $A_{ep}. N_{ep}/d$ (ha)</td>
<td>0.654</td>
<td>0.055</td>
<td>0.269</td>
<td>0.330</td>
<td>0.035</td>
<td>0.087</td>
<td>0.210</td>
</tr>
</tbody>
</table>

**TwoGener analysis**

<table>
<thead>
<tr>
<th>Differentiation in pollen gene pool among families $\Phi_h$</th>
<th>0.045</th>
<th>0.069</th>
<th>0.069</th>
<th>0.087</th>
<th>0.06</th>
<th>0.081</th>
<th>0.043</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of pollen donors $N_{ep} = (1/ 2\Phi_h)$</td>
<td>11.1</td>
<td>7.3</td>
<td>7.2</td>
<td>5.7</td>
<td>8.3</td>
<td>6.2</td>
<td>11.9</td>
</tr>
<tr>
<td>Effective pollination neighbourhood area $A_{ep}. N_{ep}/d$ (ha)</td>
<td>0.581</td>
<td>0.062</td>
<td>0.169</td>
<td>0.300</td>
<td>0.039</td>
<td>0.068</td>
<td>0.152</td>
</tr>
</tbody>
</table>
**Mating system**

Multilocus and single locus estimates of outcrossing \((t_m, t_s)\) were not significantly different from 1.0 (Table 4.2). Estimates of biparental inbreeding were low, although higher in natural sites (Table 3). Estimates for correlated paternity across all sites were similarly low too, however lowest in Neaves North, Highview Park and Jandakot restored, with corresponding higher estimates of effective numbers of pollen donors per family (Table 4.2). Overall, there were no consistent differences in any mating system parameter between restored and natural sites.

Estimates of effective number of pollen donors from MLTR and TwoGener analyses displayed a general trend of which the natural continuous site Neaves North and southern restored site Jandakot Restored having the highest number of donors and pollination neighbourhoods were consistent (Table 4.2). Pollen donor numbers differed between the two restored sites, with Gnangara having a lower number of pollen donors and pollination neighbourhood (0.087 and 0.068, respectively) than Jandakot (0.210 and 0.152, respectively). Gnangara had greater pollen pool differentiation between families than Jandakot (Gnangara restored \(\Phi_{nt} =0.081\), Jandakot restored \(\Phi_{nt} =0.043\)). The fragmented sites at Alexander and Highview had contrasting results from MLTR analysis, with Highview having a greater number effective pollen donors and a greater pollination neighbourhood than Alexander. However, TwoGener analysis produced comparative results of number of donors and pollen pool differentiation.

**Paternity assignment**

Paternity assignments were very low across the study area. Paternity was assigned to 16 offspring (2.3%) with 95% confidence and 31 offspring (4.5%) with 80% confidence. In total, 30 seeds were sired from within and 16 seeds were sired from outside the local patch. In the analysis of offspring from fragmented sites Alexander Park (AP) and Highview Park (HP), paternity of AP was assigned to 2 sired from within and 7 sired from the HP site. Paternity of HP was assigned to 3 sired from within and 2 sired from the AP site. Analysis of offspring from the Gnangara restored (GR) and adjacent Gnangara natural (GN) sites, assigned paternity of 7 GR seed to 6 sired from within and 1 sired from the GN site. Eight GN offspring were assigned, 4 sired within and 3 sired from the GR site. The analysis of Jandakot restored (JR) offspring assigned 5 sired
within and 1 sired by JN site. Three JN offspring were assigned and all 3 were sired within JN site (Table 4.3). The shortest distance of realised pollen movement was 0 m (plus or minus 4 m GPS error) the furthest was 1.33 km (Table 4.3).

**Pollen flow**

Connectivity among pollen pools and the extent to which these geographically neighbouring sites were examined through georeferenced Pollination Graphs. Successful pollen transfer (leading to viable seed production) was observed between *B. menziesii* trees across continuous habitat (NN), between fragments within an urban matrix (HP and AP) and between the two restored and adjacent sites (at Gnangara and Jandakot). The Neaves North graph contained 10 maternal individuals connected by 12 edges (Fig. 4.3A). The Alexander and Highview graph displayed 17 edges in total with 4 edge connections between sites at 3 nodes. Two Highview maternals and one Alexander maternal individual were not connected (Fig. 4.3B). Restored sites at Gnangara and Jandakot displayed contrasting pollen graphs, with Gnangara restored showing much higher connectivity with its adjacent natural site than the Jandakot restored (Fig. 4.3C and D, respectively). The Gnangara graph displayed 23 edges in total with 7 edge connections between sites at 5 nodes and with one maternal restored individual not connected (Fig. 4.3C). The Jandakot graph displayed 15 edges in total with only 2 edge connections between sites at 2 nodes (Fig. 4.3E).
Table 4.3 Simulation of paternity assignment (father given known mother) at each site under strict (95%) and relaxed (80%) confidence levels. Critical LOD (natural logarithm of the likelihood-odd ratio), number of observed assignments, assignment rate, maximum PDD (pollen dispersal distance) and Aep, estimated effective pollen area radius, centred on each maternal calculated from TwoGener results.

<table>
<thead>
<tr>
<th>Site</th>
<th>N</th>
<th>Code</th>
<th>Confidence (%)</th>
<th>Critical LOD</th>
<th>Obs. assign.</th>
<th>Assign. rate</th>
<th>Max PDD (m)</th>
<th>Aep radius (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neaves North</td>
<td>92</td>
<td>NN</td>
<td>95</td>
<td>8.20</td>
<td>2</td>
<td>2%</td>
<td>112</td>
<td>43.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
<td>6.46</td>
<td>5</td>
<td>5%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alexander</td>
<td>98</td>
<td>AP</td>
<td>95</td>
<td>8.34</td>
<td>5</td>
<td>5%</td>
<td>1320</td>
<td>14.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
<td>6.30</td>
<td>9</td>
<td>9%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Highview</td>
<td>100</td>
<td>HP</td>
<td>95</td>
<td>8.38</td>
<td>0</td>
<td></td>
<td>1331</td>
<td>23.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
<td>6.32</td>
<td>5</td>
<td>5%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gngangara natural</td>
<td>98</td>
<td>GN</td>
<td>95</td>
<td>8.38</td>
<td>1</td>
<td>1%</td>
<td>210</td>
<td>30.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
<td>6.64</td>
<td>7</td>
<td>7%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jandakot natural</td>
<td>95</td>
<td>JN</td>
<td>95</td>
<td>8.31</td>
<td>1</td>
<td>1%</td>
<td>101</td>
<td>11.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
<td>6.16</td>
<td>2</td>
<td>2%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gngangara restored</td>
<td>100</td>
<td>GR</td>
<td>95</td>
<td>8.18</td>
<td>4</td>
<td>4%</td>
<td>212</td>
<td>14.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
<td>6.17</td>
<td>3</td>
<td>3%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jandakot restored</td>
<td>98</td>
<td>JR</td>
<td>95</td>
<td>8.66</td>
<td>1</td>
<td>1%</td>
<td>159</td>
<td>21.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
<td>6.63</td>
<td>5</td>
<td>5%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 4.3 Conditional genetic covariance among pollen pools for A, continuous natural site, Neaves North; B, fragmented sites within an urban landscape, Alexander Park and Highview Park; C, Gnangara adjacent natural and restored; D, Jandakot adjacent natural and restored, depicted as Pollination Graphs overlayed on a satellite image of sites and in 3-dimensional space. Nodes represent the population of pollen haplotypes sampled by each maternal individual. Edges represent significant statistical covariance among connected pollen pools.
Pollen movement and reproductive associations

The variation in follicle production per site was explained well by pollinator visitation rate at 28% of the variation. Pollinator visitation rate by inflorescences production however, only explained 14% of the variation (Fig. 4.4). Regression analysis models testing for correlation between within population (family) pollen pool differentiation (node size produced from pollen graphs), inflorescence and follicle production revealed low but significant results, with $r^2$ values accounting for less than 20% fit of the data (a: $P < 0.001$, b: $P = 0.002$) (Fig. 4.4a,b), suggesting that the number of inflorescences may influence pollinator attraction and resulting greater differentiation in paternal contributions to family pollen pools, and number of follicles produced.

Figure 4.4 Positive associations between (a) inflorescence number and within family genetic variation (node size) and (b) follicle production and within family genetic variation (node size) in Banksia menziesii. Reproductive output was measured for each maternal tree in 2010 within each site (n = 70). Linear regression lines and the results of regression analyses are shown.
DISCUSSION

The desired outcomes for ecological restoration are typically to have rapid plant establishment, followed by functioning ecosystems for self-sustaining populations (Kettenring et al., 2014). This implies that founding individuals not only survive, but also produce enough viable offspring to grow and reproduce, sustaining the population. This study revealed that reduced potential pollen dispersal within a restoration site can reduce realised dispersal, lowering genetic differentiation among families and integration with adjacent natural sites. This is significant, as despite a high number of pollinator visitations and high levels of pollen donors within each family, comparable to (or higher than) the natural continuous site, the low complexity restored site produced fewer viable seeds.

In this study the production of *B. menziesii* inflorescences varied among sites and years. The reasons for this variation are not clearly understood across natural sites, although the below average annual rainfall in 2010 may account for decreased inflorescence production in comparison to 2011 and 2012. Despite the lower inflorescence production, this did not result in a lower inflorescence:follicle ratio. The species ability to produce follicles at a relatively high ratio in 2010 suggests that many factors, such as increased pollinator visitation due to decreased available nectar resources, priority given to reproduction in times of resource deficiency, or there may be a threshold level exists within *Banksia* (Whelan & Burbidge, 1980; Ramsay, 1989; Lamont et al., 2007). Paton and Turner (1985) indicated that *Banksia ericifolia* had a pollination threshold that can be reached within each inflorescence. Goldingay and Carthew (1998) also found that inflorescences that were open to many pollination events were found to produce a similar number of follicles to those with few pollinated florets. Reproductive variability in the species indicates its ability to produce outbred seed, despite the level of pollination events, yet still overall low seed set has been observed across many *Banksia* communities and Proteaceous species (Cowling et al., 1987; Lamont et al., 2007)

The greatest distinction between all measures of reproductive output and paternal pollen pool analyses were observed in the restored sites. The Jandakot restored site had the highest production of inflorescences and follicles. The average number of follicles and viable seed produced per maternal in Jandakot restored was also higher than all other
sites. However, the percentage of viable seeds produced per maternal was also the lowest at this site. The amount of viable seed produced following flowering is largely dependent upon resource availability (Stephenson, 1982) pollinator abundance (Paton, 1982; Wooller & Woller, 2004) and pollen quality (Ramsey & Vaughton, 2000). Previous studies in pollinator behaviour indicate that pollinators are attracted to sites with high flowering density (Paton & Ford, 1977; Paton & Turner, 1985; Armstrong, 1991b). Larger honeyeaters are known to spend more time foraging for nectar than invertebrates and smaller short-billed honeyeaters (Armstrong, 1991a; Mac Nally et al., 2005). They can also be territorial, thus dominating food resources over smaller species (Paton & Ford, 1977; Ford & Paton, 1982; Newland & Wooller, 1985; McFarland, 1986; Ramsey, 1988b; Ransay, 1989; Armstrong, 1991a; Paton, 2000; Phillips et al., 2014). The clumping of large nectar resources, such as those observed in low complexity restoration sites, have been proven to attract these dominating birds and exclude smaller birds from foraging (Comer & Wooller, 2002, Chapter 3).

Whelan and Burbidge (1980) suggests that the outcrossed breeding system of Banksia could prevent the formation or development of inbred ovules, resulting in only the production of seed derived from cross-pollination events, and overall reduced seed set. This could counteract the changed behavior of the local pollinator community. Cowling et al. (1987) demonstrated that only a small percentage of Banksia flowers develop into seed following selfing. Follicle abortion is likely to be higher among predominately outcrossing species, compared to inbreeding species, due to purging of recessive lethal alleles in the later (Levin, 1978, Schemske & Lande, 1985). Seed abortion rates were not directly measured during this study, as they are difficult to assess because the ovaries are deeply embedded in the ‘rhachis’ (or woody spine) of the inflorescence. (Goldingay & Carthew, 1998) However, studies from other banksias suggests abortion rates are high (Stock et al., 1991; Wooller & Woller, 2004; Barrett et al., 2005).

The greater flowering within the post-mine restored sites is likely due to the altered and reduced topology of the site, decreasing the distance the plants have to grow in order to access the water table. Other studies of B. menziesii observed higher follicle and seed production in road verge sites than non-verge plants, attributed to the greater availability of water and nutrients from road runoff and reduced root competition (Lamont et al. 1994 a, b). In the context of restoration, if the goal is to reflect the targeted natural
ecosystems, developing ecosystems that produce low or excessive inflorescences can alter pollinator behaviour and therefore mating system structure. However this is a developing landscape, so focus should therefore be placed on how vegetation structure is developed to create diverse and complex communities to encourage competition not only between plants but also pollinators.

Flowering intensity and site structure were shown to affect pollinator behaviour and therefore influence pollen dispersal. Fragmentation or spatial isolation does not appear to deter pollinators from moving between the Alexander and Highview sites within an urban landscape. Both sites had high levels of outcrossing, low correlated paternity and multiple shared paternal assignments, indicating they are highly connected within the landscape. Genetic isolation of these fragmented sites at this scale (less than 2 km) was not expected because the pollinators are highly mobile generalists. Furthermore, it was not expected that gene flow would be restricted between sites that were less than 150 m apart, thus the natural adjacent sites were extremely important providers of trans-boundary services into restoration sites.

Paternity assignment results and spatial differentiation of pollen pools within Jandakot natural and Jandakot restored sites shared only two significant connections among pollen pools between the sites. Complementing these results, TwoGener analysis showed Jandakot restored site had the lowest level of differentiation among pollen pool families and highest number of effective pollen donors, similar to that of the natural continuous site Neaves North. This suggests that there was a higher level of similarity between families, despite the lower levels of correlated paternity. In comparison, the Gnangara restored and natural sites had similar levels of reproductive output, differentiation among pollen pools, and multiple significant edge connections within their pollen pool networks. These results imply that there is a greater level of gene flow between these adjacent sites and successful integration of a Gnangara restored sites has been achieved. Pollination Graphs, revealed over 10 connections among pollen pools within Neaves North, while Jandakot restored maternals were not all connected suggesting smaller isolated pollen movements.

Jandakot restored and Neaves North had similar mating system results and pollen pool analyses, until the structure of each site is taken into account. Neaves North is within a
continuous site with low density of trees, the number of pollinators moving through the system is greater as the foraging behaviour must change to adapt to the distances between flowering resources. This accounts for the lower differentiation in offspring structure, correlated paternity, and highest effective pollination neighbourhood, irrespective of the higher among pollen pool connectivity. As restored populations contain higher genetic diversity with and limited to no spatial genetic structure, the effective pollination neighbourhood area in Jandakot restored is essentially very large in the available gene pool, however physically smaller. Near neighbours are likely genetically differentiated but at an increased density, so differentiated pollen movement is greater within the site.

The *B. menziesii* trees assessed were deemed to be healthy, and produced high numbers of inflorescences and follicles in both restored sites. However, restoration of functionality can only be assessed when the production of viable seed is compared. The Jandakot restored site produced fewer viable seeds in comparison to the Gnangara site. This restored population of *B. menziesii* does not appear to be as well integrated in the landscape as the northern Gnangara restored population (Fig. 4.4). We then have to investigate why this level of connectivity is not being replicated and assess what the target goals are for this site. Is its level of functioning, though at a less complex level, acceptable? Or is the goal to replicate the functioning of natural ecosystems with complex community structure? Recent reviews over a variety of ecosystems show positive correlations between biotic diversity and ecological functioning (Schwartz et al. 2000, Balvanera et al 2006, Williams 2011) and many restoration ecologists are in debate over the creation of a ‘functional ecosystem’ and what we define as a target ‘function’ (Hobbs et al. 2009).

The pollination success of a species is typically not dependent on a single pollinator species, but rather on a diverse community of pollinators (Stephan-Dewenter & Westphal 2008). The question is, if we do not create an ecologically diverse ecosystem, but create this ‘novel’ system that has altered pollinator guilds influenced by modified habitat quality, will these limited pollination services be enough to sustain the plant species populations? Unfortunately, conclusions to whether *B. menziesii* are producing enough seed to maintain viable populations cannot be made. It is not only the reproductive biology of the species that needs assessment, but the fitness of the
offspring to germinate and establish (Yates & Broadhurst 2002). Recruitment rates are generally low in banksias (Enright & Lamont, 1989; Enright & Lamont, 1992), and some species (including *B. menziesii*) require fire for seed release (Enright & Lamont, 1989), a natural element often absent from management actions in restoration sites.

It is likely that the Jandakot restored site will be sustainable, despite the decreased pollen movement distances within the site, because *Banksia* species are long-lived, predominately outcrossed and carry a high genetic load (Wiens et al., 1987). The plant-pollinator interactions examined through pollen and ecological networks display a sufficient level of functionality and robustness within the Jandakot restored site. Shorter distance mating events within this system would likely produce viable seed, as near neighbours within restored sites are more likely to be genetically unrelated than near neighbours in natural systems, a result of randomly mixing seed during collection. This production of viable seed from near neighbor mating was also displayed within seed cohorts in a restored stand of *B. menziesii* trees in (Frick et al., in press)

The role of pollination is often overlooked in restoration management (Dixon, 2009; Menz et al., 2011), yet in this study we can see how plant-pollinator interactions determine connectivity, revealing how the behavior of animals can affect ecosystem function (Lindell, 2008). Knowledge of the functional consequences of such interactions for reproduction and gene flow of plant populations are highly relevant for the long-term survival and adaptation of plant populations to these changing environments (Steffan-Dewenter & Westphal, 2008; Kaiser-Bunbury et al., 2010). Restoration programmes can be evaluated using a combined ecological and genetic approach to study networks (Forup et al., 2008, Cunningham, 2000, Ghazoul, 2005). Assessment of reproductive output, pollinator attractiveness and the dispersal of pollen through the landscape allows restoration ecologists to make practical decisions on how to create functioning ecosystems that mirror natural habitats.
Chapter 5: An evaluation of genetic diversity and spatial genetic structure in restored populations of *Banksia menziesii*

ABSTRACT

A major goal of ecological restoration is to establish self-sustaining plant populations for long-term persistence. While it is known that genetic diversity can have consequences for population fitness, the management of genetic diversity within restoration is still rarely considered. The purpose of this study was to evaluate the genetic variation and spatial genetic structure (SGS) within and among restored and natural stands of *Banksia menziesii* R.Br. (Proteaceae) in order to genetically assess restoration success. Adults and open-pollinated offspring from five natural and two restored sites of differing vegetation complexity were genotyped using 11 microsatellite DNA markers. All stands displayed high levels of genetic diversity, with restored sites having greater diversity ($A_r = 8.08; 8.34$) in comparison to natural sites ($A_r$ range = 6.49-7.41). Spatial genetic structure within all populations was weak ($Sp = < 0.007$), with significant spatial autocorrelation of genetic variation found in only one population ($r = 0.050$ at 10m; $P = 0.006$). There was significant genetic differentiation between both pairs of restored and adjacent natural sites ($F_{ST} = 0.037$ and 0.126). Source material for the highly differentiated restoration site was of unknown origin and carried a genetic signature of mixing between local and non-local alleles through a significantly higher number of alleles per locus in its offspring generation. Genetic results indicated that restored sites were established with levels of genetic diversity comparable to that of natural sites and the assessment of adjacent naturally recruited offspring revealed genetic integration. High diversity and admixture provenancing was likely the cause of the higher differentiation between the low complexity restored site and its adjacent natural site. Levels of genetic diversity and spatial structure in restored sites were equivalent to natural sites, which confirms successful management of genetic diversity through this restoration process.
INTRODUCTION

Habitat destruction is escalating worldwide with increasing anthropogenic disturbance (Ehrenfeld, 2000; Amos et al., 2012). In response, the demand for ecological restoration has rapidly grown (Broadhurst et al., 2006; Suding, 2011) with a target of restoring 150 million hectares of disturbed or degraded land globally by 2020 established by United Nations Rio+20 Conference on Sustainable Development (Merritt & Dixon, 2011; Aronson & Alexander, 2013; Menz et al. 2013). The application of genetic techniques to assess ecosystem function in ecological restoration is progressively being applied in the industry (Krauss et al., 2013; Kettenring et al., 2014). Knowledge of the appropriate level of genetic diversity and genetic composition (ie. seed sourced from one or multiple populations) used to establish and maintain ecosystem functions in restored populations remains largely undetermined (McKay et al., 2005; Forup et al., 2008; Kettenring et al., 2014). Populations with more genetic diversity are thought to have greater evolutionary resilience and long-term sustainability than that of genetically depauperate populations (Ellstrand & Elam, 1993b; Rice & Emery, 2003; McKay et al., 2005). Many studies show associations between genetic diversity and the establishment and survival of restored populations (Broadhurst et al., 2006; Sinclair & Hobbs, 2009; Bischoff et al., 2010; Breed et al., 2013; Carter & Blair, 2013). The loss of genetic diversity may cause reduced capacity to adapt to environmental change (Reed & Frankham 2003), suggesting that restored sites should be established with high genetic diversity, and reproductive functionality be restored so that these new populations maintain genetic diversity in their seed cohorts (Broadhurst et al., 2006; Sinclair & Hobbs, 2009; Bischoff et al., 2010; Breed et al., 2013; Carter & Blair, 2013).

What constitutes an appropriate choice of source of plant propagules (provenance) for ecological restoration also remains a controversial subject of vigorous debate (Hufford & Mazer, 2003; Rice & Emery, 2003; McKay et al., 2005; Broadhurst et al., 2008a; Bischoff et al., 2010; Vander Mijnsbrugge et al., 2010; Carter & Blair, 2013). The suitability of population sources for restoration is usually unknown, so ecological restoration must rely on genetic principles (Montalvo et al., 1997; Falk et al., 2006; Baer et al., 2014) or default to a ‘best guess’ of a species adaptive potential (Broadhurst et al., 2008a). The issue of restoration failure due to potential negative consequences arising from the introduction of non-local provenance genotypes has largely focused on the adaptability and fitness of the propagules (Hufford & Mazer, 2003; Broadhurst et
al., 2008; Bischoff et al., 2010; Aavik et al., 2012; Ayre et al., 2013, Baer et al., 2014). Other genetic concerns that underpin decisions in sourcing propagules include genetic swamping of local populations (Potts et al., 2003) the importance of preserving existing patterns of genetic diversity to avoid outbreeding depression and the loss of within-species diversity through the erosion of spatial genetic structure (Krauss & He, 2006). When the target species are known preferential outcrossers studying the plants genetic structure and breeding system are vital to determine its reproductive success (Coates et al., 2007; O’Brien et al., 2007; Ritchie & Krauss, 2012).

Practical guidelines indicate selection of seed from local sources (where possible), from at least 50 individuals per source population and match habitat as closely as possibly (Mortlock, 2000; Vander Mijnsbrugge et al., 2010). These guidelines are generally based on climatic or geographical separation, yet many species show a strong small-scale genetic differentiation between habitats (Vander Mijnsbrugge et al., 2010; Bradbury & Krauss, 2013). If there is a low availability of naturally sourced material, or if practitioners are constrained by time or costs, use of improper seed choice, for example from nursery planting stock, could have negative impacts to restoration success. These impacts may only become detectable after many years, especially in long-lived and slow growing plants (Vander Mijnsbrugge et al., 2010).

Since the 1980s, more attention has been focused on genetic studies of fragmented plant habitats, and the deleterious genetic effects that may occur (Lamont et al. 1993; Oostermeijer et al., 2003). Multiple studies have explored genetic structure in natural populations (Hamrick et al., 1992), however, data exist for only a fraction of the species used in restoration and few have assessed genetic variation and its spatial structure within established restored populations (Yao et al., 2010; Ritchie & Krauss, 2012). Recently more research has started to investigate fine-scale spatial structure of populations (Ishihama et al., 2005; Yao et al., 2010). Understanding spatial genetic structure (SGS) and changes in genetic diversity should help to inform decisions for management of remaining natural populations and establishment of restoration sites having important implications for the long-term survival of the population (Gapare & Aitken, 2005). SGS may be affected by a combination of factors, such as population density, breeding system, genetic drift and local selection (Wright, 1943; Vekemans &
However, limited seed dispersal within a population is considered the predominant determinant in the establishment of SGS (Jacuemyn et al., 2006).

Restored populations may decrease in viability through Allee effects, which are characterized by the disproportionate reduction in viability that occurs when a population falls below a threshold of density (Groom, 1998; Hegland & Van Leeuwen, 2001; Courchamp et al., 2008). For example, restored populations of animal-pollinated plants could undergo large reductions in seed set if pollinators are not attracted to the site, if the species are too low in density or are isolated from other patches (Groom, 1998; Courchamp et al., 2008). Gene flow can counteract these deleterious effects of genetic erosion, as in many plant species, pollen flow contributes to the maintenance of genetic variation (Levin & Kerster, 1974). Integrating restored populations with local native sites therefore becomes increasingly important when the major goal of restoration is long-term population sustainability.

In this study, I assess genetic diversity, structure and differentiation among restored and natural (fragmented and continuous) woodlands sites of the Western Australian tree species, *Banksia menziesii* R.Br (Proteaceae). *Banksia menziesii* is a keystone species of *Banksia* woodlands, which are located on the Swan Coastal Plain (SCP) within the Southwest Australian Floristic Region (SWAFR), and have undergone severe habitat destruction with urban expansion. *Banksia* woodlands are situated on significant deposits for sand mining, which have seen post-mining ecological restoration of variable standards for more than 15 years, so that many of these sites are mature and reproductively active. This provides a unique opportunity to evaluate the management and the maintenance of genetic variation not only in restored sites, and critically their offspring, for a genetic evaluation of restoration success. A greater understanding of how the natural environment and the establishment of restored sites influences genetic diversity and spatial genetic structure, could contribute to the improvement of habitat restoration efforts as well as quantify how fine-scale processes such as dispersal ultimately lead to population differentiation. Specifically, I apply microsatellite markers to address the following questions:

(i) Are restored sites of *Banksia menziesii* as genetically diverse as natural sites (fragmented and continuous)?
(ii) Are restored of sites of *Banksia menziesii* genetically divergent to their adjacent natural sites?

(iii) Are there differences in fine-scale spatial genetic structure between restored and natural sites (fragmented and continuous) of *Banksia menziesii*?

**METHODS**

*Study species and populations*

*Banksia menziesii* is a widespread tree or woody shrub species, distributed on deep sand in shrubland and woodlands of the SCP (George & Gardner, 1981; Corrick & Fuhrer, 2002) (See Chapter 2 for more details). Once a defining plant community of the SCP, only 35% of the original 6,229 km² woodland area remains, and it is being listed as a threatened ecological community. *Banksia menziesii* is a keystone species used extensively in ecological restoration of sand quarries in the region (Rokich et al., 2002).

This study was conducted in five natural and two restored *Banksia* woodland sites. A range of natural sites was selected to adequately assess the spatial variation across the reference landscape for ecological restoration evaluation. The sites included one continuous woodland site, two fragmented woodland reserves and two woodland sites adjacent to the two restored sites. The natural continuous site was located within an area of 5 Hectares within the Gnangara-Moore River State forest, Neaves North (NN). The two fragmented sites have been fragmented since about 1998 and were located within an urban landscape, Alexander (now known as Hepburn) Reserve (AP) and Highview Park (HP). The two adjacent woodland sites are neighbouring each of the two restored sites, Jandakot natural (JN) and Gnangara natural (GN). Rocla Quarry Products restored the two sites being assessed, Jandakot restored site (JR) restored in 1995 and Gnangara restored site (GR) restored in 1996 (Fig 5.1). *Banksia menziesii* plants at these sites reached reproductive maturity in 2007. Seed material used for the restoration of the GR population were apparently sourced from local provenances (Rokich, 2009) while the sources of propagules for the founding Jandakot restored population were unknown. Chapter 2 contains the full site descriptions and maps.
Figure 5.1 Aerial photographs of continuous, fragmented and restored populations of *Banksia menziesii* assessed for genetic diversity and mating pattern parameters. Each circle indicated the position of a sampled tree, White circles indicate maternal tree in which seed was collected.
Characterization of microsatellite markers and genotyping

DNA was extracted from approximately 0.8 g silica dried leaf sample (about 4 leaves) using a CTAB (cetyltrimethyl ammonium bromide)–based protocol (as modified for extracting DNA from Banksia hookeriana, Doyle & Doyle 1987, He et al. 2004). Individual seed cones were placed in a hessian bag and soaked for 1 hour, then were placed in an oven set at 150°C for 10-15 minutes until the follicles opened and seeds could be removed. Seed coats were separated from their radicles to avoid contamination of seed DNA by maternal tissue. DNA was extracted from seeds using a Jobes extraction protocol (Jobes et al. 1995). Radicles were pulverized (directly in the extraction buffer with one glass bead and 0.5 g acid washed sand in a 2 ml tube) within a tissue and cell homogenizer FastPrep®-24 instrument (MP Biomedicals, Inc., Solon, OH). The instrument was set for 24 samples of 2 ml tubes and run for 20 seconds. DNA pellets were resuspended in 75 µL of 10 mM Tris and 1 mM EDTA, and 2 µL (of 5µg DNA/100µl dH₂O) of this mixture was used in each polymerase chain reaction (PCR). Microsatellite amplifications were performed for eleven polymorphic markers (BmA1, BmB6, BmB102, BmB106, BmC2, BmD1, BmD4, BmD103 and BmD105) previously designed for B. menziesii (He 2013) and two (BaA3, BaB106) previously designed for B. attenuata (He et al. 2004; 2007). Amplifications were performed with the following PCR conditions: 96°C for 2 min (1 cycle), followed by 30 cycles of 94°C for 1 min, 52 to 57°C for 1 min (according to each primer pair annealing temperature, Appendix 2), 72°C for 1 min and final extension time of 72°C for 7 min. PCR products were stored at 4°C then purified with an isopropanol clean-up (Appendix 2) before being separated by capillary electrophoresis using a Beckman Coulter CEQ 8800 Genetic analysis system. An internal size standard (SS400) was added to each well loaded on the CEQ 8800. Alleles were scored using the CEQ 8800 Genetic Analysis System program.

DATA ANALYSIS

Genetic diversity within sites and site differentiation

The program Micro-Checker (van Oosterhout et al., 2004) was used to ensure that the data from each locus matched the simple sequence repeats for the individual microsatellites, and outliers and possible presence of null alleles were identified. Population genetic measures; number of alleles (Na), effective number of alleles (Ne),
Chapter 5: Genetic diversity and structure

number of private alleles ($P$), observed heterozygosity ($H_o$), expected heterozygosity ($H_e$), Fixation indices ($F_{IS}$), Shannon’s information index ($I$) was used to measure genetic diversity of the populations. The program FSTAT (Goudet, 2001) was used to estimate genetic measures of allelic diversity, allelic richness ($A_r$) (equivalent of $H_e$) that are corrected for samples of different sizes, allowing comparison among populations. For each measure of genetic diversity, its mean and 95% confidence intervals were calculated. Significant differences between parameter estimates were declared if their 95% confidence interval did not overlap. An analysis of molecular variance (AMOVA) and pair-wise population differentiation ($F_{ST}$) was performed to partition the total genetic variation into within and among natural and restored site components using Arlequin ver 3.4.1.2 (Excoffier et al., 2005).

**Fine-scale spatial genetic structure**

Spatial genetic structure (SGS) within each site was estimated by spatial autocorrelation analysis within GenAlEx 6.5 (Peakall & Smouse, 2012), using distance classes of 10 m with 12 classes in total to assess the genetic correlation among individuals as a function of geographic distance. Spatial structure graphs (correlograms) were produced for all seven sites with calculated correlation coefficient $r$ and confidence limits, as generated by 999 random permutations of the data and bound by the 95% confidence interval about the null hypothesis of no spatial structure (Peakall & Smouse 2012).

To further assess overall spatial genetic structure in each site the $Sp$ statistic was used, which directly quantifies comparisons of the magnitude of spatial genetic structure among populations (Vekemans & Hardy, 2004). The $Sp$ statistic was calculated using kinship coefficients determined for each locus $Sp = -b(1 - F_{1})$, where $b$ is the regression slope of $F_{ij}$ on ln (distance); $F_{1}$ is the mean $F_{ij}$ value between individuals belonging to the first distance interval that should include all pairs of neighbours (Ritland, 1996; Jones & Hubbell, 2006), and all loci combined within each site. This was performed with 10000 permutations using SPAGeDi 1.3 (Hardy & Vekemans, 2002). The inverse of $Sp$ was calculated as an indirect estimate of Wright’s neighbourhood size $Nb$ ($Nb = 1/Sp$) for each site.
RESULTS

Genetic diversity within sites

Of the 710 adult trees for which DNA was extracted, 701 were genotyped at eleven microsatellite markers. The remaining 9 samples were eliminated from analyses as they failed to consistently amplify markers. There was no significant difference for mean expected heterozygosity \((H_e; F = 0.38, df = 6, P = 0.89)\) and mean observed heterozygosity \((H_o; F = 0.60, df = 6 P = 0.73)\) between all sites of adults and between site classification (Table 5.1). Fixation indices \((F_{IS})\) between all adult \((F_{IS}; F = 2.48, df = 6, P = 0.03)\) sites differed significantly, produced from the genetic differentiation of the Jandakot Restored and low differentiation in the Gnangara Restored site. The average number of alleles per locus \((N_a)\) in the restored Gnangara and Jandakot sites \((N_a \pm SE = 9.36 \pm 1.56; n = 101; N_a \pm SE = 9.41 \pm 1.25; n = 99, respectively)\) were not significantly different from their adjacent natural sites \((GN: 7.64 \pm 1.16; n = 94; JN: 6.82 \pm 1.30)\) and the Neaves North site \((7.46 \pm 1.19; n = 96)\) \((F= 0.57, df =4, P = 0.69)\).

The effective number of alleles \((N_e)\) \((N_e; F = 0.14, df =6, P = 0.99)\) and Shannon’s Information Index \((I)\) \((I; F = 0.31, df = 6, P = 0.93)\) was not significantly different between adults. The mean number of private alleles \((P_r)\) in adult trees was greater in both restored sites \((GR: P_r = 0.64 \pm 0.203, JR: P_r = 0.64 \pm 0.364)\) than the natural sites \((fragmented and continuous)\) \((P_r range = 0.90 - 0.27)\) (Table 5.1, Fig. 5.2). Allelic richness was not significantly different across all adults and offspring \((A_r: F=0.45, df =13, P = 0.95)\), however, tended to be higher in both restored sites and increased within offspring of Jandakot Natural (Table 5.1).

Of the 700 offspring for which DNA was extracted, 687 were genotyped, as 13 failed to consistently amplify markers. There was no significant difference between the offspring of natural, fragmented and restored sites for expected and observed heterozygosity \((H_e; F = 0.23, df =6, P = 0.96; H_o; F = 0.15, df =6, P = 0.99)\) (Table 5.1). The effective number of alleles \((N_e)\) \((N_e; F = 0.21, df =6, P = 0.97)\) and Shannon’s Information Index \((I)\) was not significantly different between offspring cohorts \((I; F = 0.21, df = 6, P = 0.97)\) (Table 5.1, Fig. 5.2). Mean number of private alleles within offspring cohorts were not significantly different across sites, however, Alexander and Neaves North maintained, Highview Park, Gnangara restored and Jandakot restored lost, and both adjacent sites Gnangara and Jandakot increased in private allele frequency.
Table 5.1 Genetic diversity measures (95% confidence intervals in parentheses) in sites of *Banksia menziesii*.

<table>
<thead>
<tr>
<th>Site</th>
<th>Code</th>
<th>Type</th>
<th>N</th>
<th>Ho</th>
<th>He</th>
<th>$F_{st}$</th>
<th>Na</th>
<th>Ne</th>
<th>I</th>
<th>Pr</th>
<th>Ar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alexander Park</td>
<td>AP</td>
<td>Fragmented</td>
<td>92</td>
<td>0.44</td>
<td>0.57</td>
<td>0.22</td>
<td>7.09</td>
<td>3.29</td>
<td>1.20</td>
<td>0.18</td>
<td>6.49</td>
</tr>
<tr>
<td>Highview Park</td>
<td>HP</td>
<td>Fragmented</td>
<td>99</td>
<td>0.46</td>
<td>0.62</td>
<td>0.27</td>
<td>8.09</td>
<td>3.77</td>
<td>1.35</td>
<td>0.27</td>
<td>7.41</td>
</tr>
<tr>
<td>Neaves North</td>
<td>NN</td>
<td>Continuous</td>
<td>96</td>
<td>0.52</td>
<td>0.58</td>
<td>0.10</td>
<td>7.46</td>
<td>3.46</td>
<td>1.24</td>
<td>0.18</td>
<td>6.73</td>
</tr>
<tr>
<td>Gnangara natural</td>
<td>GN</td>
<td>Adjacent</td>
<td>105</td>
<td>0.48</td>
<td>0.53</td>
<td>0.10</td>
<td>7.64</td>
<td>3.01</td>
<td>1.16</td>
<td>0.09</td>
<td>6.66</td>
</tr>
<tr>
<td>Jandakot natural</td>
<td>JN</td>
<td>Adjacent</td>
<td>109</td>
<td>0.50</td>
<td>0.54</td>
<td>0.10</td>
<td>6.82</td>
<td>2.78</td>
<td>1.01</td>
<td>0.18</td>
<td>6.66</td>
</tr>
<tr>
<td>Gnangara restored</td>
<td>GR</td>
<td>Restored</td>
<td>101</td>
<td>0.55</td>
<td>0.57</td>
<td>0.05</td>
<td>9.36</td>
<td>3.47</td>
<td>1.28</td>
<td>0.64</td>
<td>8.08</td>
</tr>
<tr>
<td>Jandakot restored</td>
<td>JR</td>
<td>Restored</td>
<td>99</td>
<td>0.48</td>
<td>0.67</td>
<td>0.30</td>
<td>9.46</td>
<td>3.83</td>
<td>1.44</td>
<td>0.64</td>
<td>8.34</td>
</tr>
</tbody>
</table>
Table 5.1 Cont. Genetic diversity measures (95% confidence intervals in parentheses) in sites of *Banksia menziesii*.

<table>
<thead>
<tr>
<th>Site</th>
<th>Code</th>
<th>Type</th>
<th>N</th>
<th>Ho</th>
<th>He</th>
<th>Na</th>
<th>Ne</th>
<th>I</th>
<th>Pr</th>
<th>Ar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Offspring</td>
<td>AP</td>
<td>Fragmented</td>
<td>99</td>
<td>0.51</td>
<td>0.53</td>
<td>(6.91)</td>
<td>(2.90)</td>
<td>(1.10)</td>
<td>(0.18)</td>
<td>(6.11)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.44-0.58)</td>
<td>(0.46-0.60)</td>
<td>(5.65-8.17)</td>
<td>(2.27-3.53)</td>
<td>(-0.09-1.29)</td>
<td>(0.06-0.30)</td>
<td>(5.01-7.21)</td>
</tr>
<tr>
<td>Offspring</td>
<td>HP</td>
<td>Fragmented</td>
<td>100</td>
<td>0.49</td>
<td>0.52</td>
<td>7.55</td>
<td>3.15</td>
<td>1.12</td>
<td>0.18</td>
<td>6.41</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.41-0.57)</td>
<td>(0.45-0.59)</td>
<td>(6.15-8.95)</td>
<td>(2.32-3.98)</td>
<td>(0.92-1.22)</td>
<td>(0.06-0.30)</td>
<td>(5.23-7.59)</td>
</tr>
<tr>
<td>Offspring</td>
<td>NN</td>
<td>Continuous</td>
<td>98</td>
<td>0.51</td>
<td>0.52</td>
<td>7.09</td>
<td>2.78</td>
<td>1.10</td>
<td>0.18</td>
<td>6.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.45-0.57)</td>
<td>(0.46-0.58)</td>
<td>(5.93-8.25)</td>
<td>(2.22-3.34)</td>
<td>(0.93-1.27)</td>
<td>(0.06-0.30)</td>
<td>(5.28-7.36)</td>
</tr>
<tr>
<td>Offspring</td>
<td>GN</td>
<td>Adjacent</td>
<td>105</td>
<td>0.48</td>
<td>0.53</td>
<td>7.64</td>
<td>3.01</td>
<td>1.16</td>
<td>0.09</td>
<td>6.66</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.42-0.54)</td>
<td>(0.46-0.60)</td>
<td>(6.48-8.80)</td>
<td>(2.27-3.75)</td>
<td>(0.97-1.35)</td>
<td>(0-0.18)</td>
<td>(5.57-7.75)</td>
</tr>
<tr>
<td>Offspring</td>
<td>JN</td>
<td>Adjacent</td>
<td>95</td>
<td>0.51</td>
<td>0.48</td>
<td>9.41</td>
<td>3.83</td>
<td>1.44</td>
<td>0.20</td>
<td>6.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.44-0.58)</td>
<td>(0.41-0.55)</td>
<td>(8.18-10.64)</td>
<td>(3.09-4.57)</td>
<td>(1.29-1.59)</td>
<td>(0.19-0.28)</td>
<td>(5.77-8.11)</td>
</tr>
<tr>
<td>Offspring</td>
<td>GR</td>
<td>Restored</td>
<td>100</td>
<td>0.53</td>
<td>0.55</td>
<td>8.18</td>
<td>3.24</td>
<td>1.20</td>
<td>0.18</td>
<td>7.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.45-0.61)</td>
<td>(0.48-0.62)</td>
<td>(6.81-9.55)</td>
<td>(2.49-3.99)</td>
<td>(1.00-1.40)</td>
<td>(0.06-0.30)</td>
<td>(5.83-8.27)</td>
</tr>
<tr>
<td>Offspring</td>
<td>JR</td>
<td>Restored</td>
<td>97</td>
<td>0.55</td>
<td>0.60</td>
<td>7.46</td>
<td>3.63</td>
<td>1.29</td>
<td>0.36</td>
<td>6.86</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.49-0.61)</td>
<td>(0.54-0.66)</td>
<td>(6.27-8.65)</td>
<td>(2.71-4.55)</td>
<td>(1.10-1.48)</td>
<td>(0.16-0.56)</td>
<td>(5.72-8.00)</td>
</tr>
</tbody>
</table>

First row of sites are adults, second row are offspring. Type indicates site landscape. N, number of samples; Ho, average observed heterozygosity; He, average expected heterozygosity adjusted for sample size; Na, average number of alleles per locus; Ne, effective number of alleles; I, Shannon’s Information Index; Pr, number of private alleles; Ar, and mean allelic richness adjusted for sample size.
Figure 5.2 Allelic diversity and heterozygosity across sites of *Banksia menziesii* adults and offspring. *Na*, average number of alleles per locus; *Ne*, effective number of alleles; *I*, Shannon’s Information Index; *He*, average expected heterozygosity. AP, Alexander Park; HP, Highview Park; NN, Neaves north; GN, Gnangara natural; JN, Jandakot natural; GNangara restored and JR, Jandakot restored.
**Site differentiation**

AMOVA partitioned 94.7% of the total variation within and 5.3% among the adults of the Gnangara natural and restored site (Table 5.2) and 97.7% within and 2.3% among their offspring (Table 5.2). Differentiation between Jandakot natural (JN) and restored (JR) adults was 88.8% within and 11.2% among and their offspring 90.4% within and 9.6% among (Table 5.2). Adults and offspring in both northern (Gnangara) and southern (Jandakot) sites demonstrated a low, but significantly greater than zero, degree of gene differentiation among sites in terms of allele frequencies (Gnangara: $F_{ST} = 0.053$, $P < 0.0001$ (adults) and $F_{ST} = 0.002$, $P < 0.0001$ (offspring); Jandakot: $F_{ST} = 0.111$, $P < 0.0001$ (adults) and $F_{ST} = 0.151$, $P < 0.0001$ (offspring)). Similarly, there was significant gene differentiation between adults from the fragmented (Alexander and Highview) sites ($F_{ST} = 0.044$, $P = 0.001$). Average pairwise site genetic differentiation of the Jandakot restored site to all other sites (mean $F_{ST} = 0.060$; $n = 6$) was twice that of all other pairwise comparisons (mean $F_{ST} = 0.028$; $n = 15$).
Table 5.2 Analysis of molecular variance (AMOVA) for adult and offspring cohorts of *Banksia menziesii*. Variance was calculated between one restored site and one natural site in Gnangara and in Jandakot, and between natural sites, using 11 polymorphic microsatellite markers.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>SSD</th>
<th>Variance Component</th>
<th>% of Total</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gnangara adults; natural/ restored</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among</td>
<td>1</td>
<td>14.24</td>
<td>0.06</td>
<td>5.29</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Within</td>
<td>205</td>
<td>467.51</td>
<td>1.14</td>
<td>94.71</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>206</td>
<td>481.76</td>
<td>1.20</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td>Jandakot adults; natural/ restored</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among</td>
<td>1</td>
<td>15.15</td>
<td>0.07</td>
<td>11.15</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Within</td>
<td>207</td>
<td>231.95</td>
<td>0.56</td>
<td>88.85</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>208</td>
<td>247.09</td>
<td>0.63</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td>Gnangara offspring; natural/ restored</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among</td>
<td>1</td>
<td>5.69</td>
<td>0.02</td>
<td>2.31</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Within</td>
<td>204</td>
<td>394.24</td>
<td>1.00</td>
<td>97.69</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>205</td>
<td>399.93</td>
<td>1.02</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td>Jandakot offspring; natural/ restored</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among</td>
<td>1</td>
<td>13.07</td>
<td>0.07</td>
<td>9.61</td>
<td>0.001</td>
</tr>
<tr>
<td>Within</td>
<td>191</td>
<td>142.11</td>
<td>0.37</td>
<td>90.39</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>192</td>
<td>155.18</td>
<td>0.43</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td>Fragmented; Alexander Park/Highview Park</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among</td>
<td>1</td>
<td>35.12</td>
<td>0.17</td>
<td>4.42</td>
<td>0.001</td>
</tr>
<tr>
<td>Within</td>
<td>380</td>
<td>409.5</td>
<td>3.58</td>
<td>95.58</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>381</td>
<td>444.62</td>
<td>3.74</td>
<td>100.00</td>
<td></td>
</tr>
</tbody>
</table>

Statistics include sums of squared deviations (SSD), variance component estimates, the percentage of the total variance (% total) contributed by each component, and the probability (P) of obtaining a more extreme component estimate by chance alone.
Fine-scale spatial genetic diversity within populations

Spatial autocorrelation analysis identified significant genetic structure at an interplant distance of 10 m within the Gnangara natural site ($r = 0.050; P = 0.006$; Fig. 5.2). No significant spatial genetic structure was found for any other distance class in any site. The $Sp$ statistic was very low in all sites ($Sp = < 0.007$), with Jandakot restored showing no spatial structure ($Sp = -0.0002$). The estimates of neighborhood size ($Nb$) ranged from 144 to 286 trees in the continuous and fragmented sites, while the Gnangara restored site (GR) had the largest $Nb$ of 401 (Table 5.3) and Jandakot restored was undefined.

Table 5.3 Spatial genetic structure measures; $Sp$ statistic and Neighbourhood size ($Nb$) for sites of *Banksia menziesii*

<table>
<thead>
<tr>
<th>Site</th>
<th>Site type</th>
<th>Sp statistic</th>
<th>Neighbourhood size (Nb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alexander</td>
<td>Fragmented</td>
<td>0.0035</td>
<td>285</td>
</tr>
<tr>
<td>Highview</td>
<td>Fragmented</td>
<td>0.0064</td>
<td>156</td>
</tr>
<tr>
<td>Neaves North</td>
<td>Continuous</td>
<td>0.0028</td>
<td>361</td>
</tr>
<tr>
<td>Gnangara Natural</td>
<td>Adjacent</td>
<td>0.0069</td>
<td>144</td>
</tr>
<tr>
<td>Jandakot Natural</td>
<td>Adjacent</td>
<td>0.0035</td>
<td>286</td>
</tr>
<tr>
<td>Gnangara Restored</td>
<td>Restored</td>
<td>0.0025</td>
<td>401</td>
</tr>
<tr>
<td>Jandakot Restored</td>
<td>Restored</td>
<td>-0.0002</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 5.3 Spatial autocorrelation analysis correlograms for populations of *Banksia menziesii*, showing the genetic correlation coefficient ($r$) for increasing distance class sizes, with 95% confidence intervals about $r$ as determined by bootstrapping (in red). (A, Alexander Park; B, Highview Park; C, Gnangara natural; D, Gnangara restored; E, Jandakot natural; F, Jandakot restored and G, Neaves North).
DISCUSSION

This assessment of genetic diversity and spatial genetic structure (SGS) across multiple populations and generations of *Banksia menziesii* has shown that restored populations were equivalent to or greater than natural sites for at least the first 15 years following establishment. Comparisons among the restored site seed cohorts indicated that diversity was maintained at a level comparable to that of the natural site offspring. Jandakot restored site was genetically differentiated from its adjacent natural site indicating that the founding seeds for this restoration were likely sourced from non-local provenances. Differences in the change of genetic diversity between the adults and their offspring for restored and adjacent natural populations reflect high genetic connectivity among these sites. Surprisingly for a species with primarily gravity-dispersed seed, little evidence of fine-scale SGS was found in any natural site. Self-incompatibility, lack of recruitment and the age structure of the trees are all driving forces counteracting fine-scale SGS and preventing erosion of genetic diversity for future generations, and as a result, no significant differences between sites were found.

Allelic richness and the number of private alleles were higher in restored populations than natural populations. In contrast, Broadhurst (2013) found significantly lower genetic diversity in restored sites of *Eucalyptus melliodora* than in natural sites. Seed sourcing methods have direct impacts on the allelic richness and gene diversity of restored populations. The presence of high allelic richness and private alleles in the restored *Banksia menziesii* populations indicates that the seed was sourced from multiple populations. The degree of genetic differentiation of the Jandakot site from all other sites, compared to that of the Gnangara restored site, indicates multiple non-local provenance sources for the former, and multiple local provenance sources for the latter (Krauss et al. 2013).

Genetic diversity at restored sites will be affected by gene flow with adjacent natural sites (Travis et al., 2002; Liu et al., 2008). Pollen movement (Chapter 4) between restored and natural trees has resulted in the introgression of introduced alleles into the offspring of adjacent natural site. The increased allelic richness, within the Jandakot natural offspring cohort (Table 5.1; Fig. 5.2) confirms genetic exchange between *B. menziesii* at restored and natural sites.
Reduced differentiation in relation to fixation \((F_{ST})\) between Jandakot offspring further suggests genetic connectivity among restored and adjacent natural sites. The consequences of new non-local alleles from the restored site population entering and mixing with substantially differentiated local alleles introgressing with the local gene pool are still unknown—could be beneficial (heterosis) or detrimental (outbreeding depression) (Hufford & Mazer 2004; McKay et al. 2005). However, in *B. ilicifolia*, wide outcrossing by hand of plants separated by 30 km leads to heterosis, expressed as improved germination of seed and early growth of seedlings (Heliyanto et al. 2006). This suggests that wide outcrossing stemming from the introduction of non-local provenance genotypes in banksias are more likely to be beneficial than detrimental, but does require further investigation. The Jandakot site offers a unique opportunity for this on-going assessment.

All but one natural site indicated signs of fine-scale SGS within 10 m, demonstrating there are ecological and genetic factors countering the immediate effect of primarily gravity dispersed seeds (Krauss et al. 2009). In support, \(Sp\) values were much lower than the average statistic \((Sp = 0.030)\) reported for 47 plant species in Vekemans and Hardy (2004) and other woody long-lived trees with animal-pollinated outcrossing breeding systems and gravity dispersed seeds. SGS can arise from a variety of factors, however, gene flow, through pollen and seed dispersal is a key determinant in establishment. An absence of fine-scale structure within restored sites is a result of seed establishment techniques used in restoration that effectively randomise SGS (i.e. return of stripped soil seed bank, random application of broadcast seed, and random planting of green stock), with no natural recruitment in the sites. The absence of SGS in restored sites has important genetic implications or restoration, as it increases seed sourcing potential and does not require focus in restored population establishment. However, SGS would be expected in natural sites as exhibited in *Banksia attenuata* (Ritchie & Krauss 2012) and *B. hookeriana* (Krauss et al., 2009). The weak SGS of the *B. menziesii* natural sites may result from a combination of factors such as mature long-lived trees, low population density due to thinning (Jacquemyn et al., 2006) (natural and unnatural due decreased water table and disease), weak serotiny (canopy-stored seed) (George, 1980), wide-outcrossing and low bi-parental inbreeding (see Chapter 4), low seed set (Whelan & Burbidge, 1980), short-lived seeds that are therefore absent from a persistent seed bank and low recruitment rates due in part to an absence of fire (Cowling & Lamont, 1987).
As a consequence of summer flowering, *B. attenuata* is visited on average by a higher percentage of insects, and is highly serotinous (George, 1980) with viable seed held in cones for at least 10 years (Cowling et al., 1987), conceivably the reason why SGS was found (Ritchie & Krauss 2012; see Chapter 6). Both species are linked to fire for seed release, recruitment and establishment (Cowling et al., 1987). The lack of fire in these sites (since 1970) may be a controlling factor in limiting seed dispersal and seedling establishment and needs further investigation.

Knowledge of fine-scale SGS of populations can aid restoration and seed collecting practitioners for sampling strategies that most efficiently avoid closely related seed sources (Cavers et al., 2005). For example, Yao et al. (2010) advised that due to a presence of strong family clustering within fragmented populations of *Sinojackia reheriana* (Styracaceae), seed for ex-situ conservation should be collected from trees at a distance of 19m to reduced genetic similarity between neighbor individuals. Results from an analysis of SGS in *Eucalyptus incrassata* (Myrtaceae) suggests individuals are more closely related within 50 m than beyond 50 m (Breed et al., 2012) and for the avoidance of inbreeding, restoration seed collections should target trees beyond 50m. While SGS in *B. menziesii* was overall weak, SAA detected weak but significant SGS at 10m, but not beyond, in some natural populations. Consequently, the recommendation from this study is that seed collection from trees separated by > 10m avoids collection from genetically related trees. The very weak SGS within natural sites is fortuitous for establishing restored sites, as fine-scale SGS does not seem to play a vital role in ecosystem functioning. A further consequence for seed collection is that fewer trees (>10m apart) are required as seed sources because their seed contain much of the genetic diversity within a population due to near random mating. As these species display near random mating, the potentially negative genetic consequences of collecting seed from fewer trees are reduced.

This study of multiple sites across varying landscapes containing *Banksia menziesii* joins others (Coates & Sokolowski 1992; Coates et al 2007; Krauss et al., 2009; Ritchie & Krauss 2012; Llorens et al., 2013; Frick et al. in press), to show that banksias typically contain high genetic loads, weak SGS and wide effective gene flow through highly mobile pollinators. Pollinators of these species, with their foraging range and
mobility breakdown any isolation effects of the fragmented sites studied. With greater isolation, genetic connectivity would likely decrease. Krauss et al. (2013) suggested a radius up to 30 km from the restoration site is adequate for local seed provenance collection of *B. menziesii*. As the reproductive success of banksias relies upon the successful delivery of outcrossed pollen by animal-pollinators (highly mobile birds and insects) (Scott, 1980; Whelan & Burbidge, 1980; Collins & Rebelo, 1987; Ritchie & Krauss, 2012) pollen dispersal distances in *Banksia menziesii* are likely to be great (see Krauss et al., 2009) and gene flow extensive.

The current study indicates that natural remnants have comparable levels of genetic diversity to larger, intact populations, with no evidence for inbreeding or genetic isolation effects. These results suggest that remnants of *B. menziesii* are adequate sources for seed collection for restoration, and that the assumption of physical site size correlates to the functioning population size is incorrect. Recent restoration guidelines recommend against the use of seed from small populations, even when local (Broadhurst et al. 2008). The results of the current study show that *B. menziesii* populations effectively extend beyond the physical fragment limits, and as a consequence, seed from these sites are not genetically inferior.

This study confirmed the initial sourcing of genetically diverse propagules, reflective of the levels within natural sites, and the maintenance of this genetic diversity within seed cohorts. There was evidence of genetic differentiation between restored and natural adjacent sites and genetic integration with these adjacent natural sites. Effects of this integration require further investigation as the possible negative effects such as outbreeding depression, or positive effects such as heterosis, may result (Hufford and Mazer 2003; Broadhurst et al. 2006; Bischoff et al. 2010; Frankham et al. 2011; Aavik et al. 2012).
Chapter 6: An evaluation of genetic diversity and reproductive output in restored populations of *Banksia attenuata*

**ABSTRACT**

Ecosystem functionality is influenced by plant community species diversity. However, the consequence of initial species diversity for ecological restoration success is poorly understood. This study assesses key components of reproductive functionality, adult and offspring genetic diversity, spatial genetic structure (SGS), mating patterns, effective pollen dispersal and reproductive output, in a low species diversity restored site and its adjacent natural site, for the dominant keystone species *Banksia attenuata* R.Br. (Proteaceae). The data are then compared with those for a high species diversity restoration site to assess the impact of initial plant species diversity on restoration success. For *B. attenuata* allelic richness across 8 microsatellite loci was higher in the restored sites (low diversity site $Ar = 11.05$, high diversity site $Ar = 8.76$) than their adjacent natural sites (low, high: $Ar = 8.33, 7.03$) while heterozygosity was similar for adults and offspring (low, high: $He$ range = 0.66 – 0.69, 0.57–0.62). Fruit (follicle) set was significantly lower in the low diversity restored site than in the high diversity restored site. All sites contained weak SGS (Restored low, high: $Sp = 0.001, 0.002$; Natural low, high: $Sp = 0.002, 0.006$). Genetic divergence from its adjacent natural site was much greater for the low complexity restored site ($F_{ST} = 0.071$) than for the high complexity restored site ($F_{ST} = 0.006$). At all restored and adjacent natural sites, *B. attenuata* was completely outcrossing with low correlated paternity ($r_p = 0.061-0.159$). Trees in adjacent natural sites sired 41% of offspring of trees in the restored site and 49% of assigned offspring in the natural site were sired by trees from the restored site. Consequently, despite differences in species diversity of restored communities there was no difference in the delivery of pollinator services and reproductive functionality for *B. attenuata*. Genetic integration through pollen dispersal was facilitated between natural and restored sites. However, the long-term consequences of using non-local provenance seed in the low diversity restored site requires further investigation.
INTRODUCTION

Ecological restoration is the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed (SER, 2004). Due to the widespread need to restore and the limited knowledge and/or funds typically available for natural resource management, there are still doubts surrounding the effectiveness of restoration programs (Hobbs & Cramer, 2008; Suding, 2011; Wortley et al., 2013). Despite being a crucial element of assessing or evaluating ecological restoration success, a number of projects fail to define targets and lack sufficient monitoring (Palmer et al., 1997; Devoto et al., 2012). This is partly due to the lack of knowledge in restoration ecology as the discipline is still young (Young et al., 2005) and also timescale ecological processes take to develop, are typically outside the realm of individual restoration projects (Ehrenfeld, 2000). There is still extensive debate on what characterizes successful restoration and how we should best evaluate and measure restoration outcomes (Suding, 2011; Wortley et al., 2013). Practitioners often endeavor to match natural pre-disturbance conditions as a standard of restoration success (using a natural reference site) and the primary focus is to restore communities with its particular associated organisms (Ehrenfeld, 2000; Hobbs & Cramer, 2008).

Ecologists widely accept that there is a direct relationship between ecosystem structure and function, and given its importance and implications for ecosystem restoration, it is not surprising that a large number of conceptual models have been developed to describe this association (Hobbs & Norton, 1996; Cortina et al., 2006; Fortuna & Bascompte, 2006). Ecological or ecosystem functions are the attributes of ecosystems, including the interactions among organisms and between organisms and their environment (SER, 2004). These ecological models are used to aid restoration project planning and are applied to predict the outcomes of restoration (Anand & Desrochers, 2004; Cale et al., 2009). These models and frameworks are based on the principles that reduced ecosystem structure and composition will reduce ecosystem functioning (Bartha et al., 2004; Cortina et al., 2006; Suding & Hobbs, 2009). However, there have been few attempts to test this, empirically (Lindenmayer et al., 2008).

Pollen dispersal is an ecosystem process that is greatly influenced by the landscape and the interacting ecological attributes within (Sork et al., 1999). A key component of ecosystem functionality for outcrossing plant species is the delivery of robust pollinator
services that reduce inbreeding and produce outbred offspring (Moeller, 2004). In doing so, the species achieves genetic self-sustainability and resilience through the avoidance of an erosion of genetic diversity. Animal-pollinated plants in restoration are reliant on their pollinators to move into the restored landscape (Amarasekare, 2004; Knight et al., 2005), and pollinator and plant diversity are often correlated (Ghazoul, 2006; Ebeling et al., 2008).

Vegetation structure and species composition used in a restoration project can indirectly affect the behaviour of pollinators, influence pollen flow, and therefore mating systems (Cunningham, 2000; Ghazoul, 2005). Following changes in the spatial distribution of populations (e.g. population size and plant density), pollinator behaviour, diversity and abundance can change greatly (Steffan-Dewenter & Westphal, 2008). Founding a restored site with a low density of reproductive plants, (i.e. the number of plants available for potential mating, or the plant pollinator neighbourhood (Wright, 1969; García-Robledo, 2010), could alter foraging and flight movements of pollinators (Aizen & Feinsinger, 1994; Quesada et al., 2004), and reduce pollinator attraction (Bond, 1994; Kearns et al., 1998; Wilcock & Neiland, 2002; Knight et al., 2005). If pollinators avoid restored sites, pollen flow is altered, resulting in potential reductions of pollen flow with adjacent habitats, reduced seed set, fewer seedlings, increased population structure and increased inbreeding (Ghazoul, 2005; García-Robledo, 2010; Anderson et al., 2010). Knowledge of how genetic structure and inter-specific interactions such as plant-pollinator relations are restored (Care et al. 2006) or how restoration can alter pollination systems is, however, still in its infancy (Ghazoul, 2005; García-Robledo, 2010; Menz et al., 2011). The implementation of a genetic assessment using molecular markers to identify plant spatial genetic structure and contemporary mating systems of natural and restored systems addresses these gaps (Ritchie & Krauss, 2012; Williams et al., 2014).

Restoring population connectivity and integrating the restored site into the surrounding landscape is a fundamental component of ecological restoration (McKay et al., 2005; Suding, 2011). Poor choice of source plant material could potentially result in poor integration with surrounding vegetation (Bussell et al., 2006). Previously research has focused on genetic differentiation and adaptation of propagules used for restoration, now investigation into selecting plants for restoring function, such as, to promote and
restore pollinators is increasing, with a focus on restoring plants that attract and sustain pollinators (Dixon, 2009; Menz et al., 2011). Plant species that produce nectar and/or pollen to support pollinator communities are referred to as ‘framework’ species (Dixon, 2009) or ‘interactor’ species (Palmer et al., 1997; Menz et al., 2011). These plant species might be able to sustain a pollinator community that would also serve to facilitate the pollination to other plant community species that may not be as attractive (Ghazoul, 2006; Molina-Montenegro et al., 2008; Lázaro et al., 2009). Facilitating this connectivity, through gene flow between restored and adjacent natural sites needs consideration when initially selecting source material for establishing the restoration site (Hufford & Mazer, 2003; McKay et al., 2005; Aavik et al., 2012). Seed provenance and genotypic diversity have been documented to have strong association, affecting the performance of the founding population (Sackville Hamilton, 2001; Hufford & Mazer, 2003; Bischoff et al., 2010). Restoration is most likely to be successful if source material is of high fitness, retaining maximum genetic diversity, free from the deleterious effects of inbreeding (Charlesworth & Charlesworth, 1987; Broadhurst et al., 2008a) and is suitably adapted to the local restoration site conditions (Hufford & Mazer, 2003; McKay et al., 2005; O'Brien et al., 2007).

Seed provenance and genotypic diversity has an affect on the performance of the founding population (Sackville Hamilton, 2001; Hufford & Mazer, 2003; Bischoff et al., 2010). Restoration is most likely to be successful if source material is of high genetic diversity, free from the deleterious effects of inbreeding (Charlesworth & Charlesworth, 1987; Broadhurst et al., 2008a) and is suitably adapted to the local restoration site conditions (Hufford & Mazer, 2003; McKay et al., 2005; O'Brien et al., 2007). Using local provenance seed is in most practice guidelines (Mortlock, 2000; McKay et al., 2005; Bussell et al., 2006; O'Brien et al., 2007), as local genotypes when grown in their home site should perform better than distant genotypes, (the “home-site advantage” hypothesis) and is well documented for many plants (Bradshaw, 1984; Linhart & Grant, 1996; Galloway & Fenster, 2000; Montalvo & Ellstrand, 2000; 2001; Bradley St Clair et al., 2013). However, doing so does not guarantee best establishment or performance as most restoration sites differ from their undisturbed reference (or pre-disturbance) sites, but it does reduce the risk of introducing harmful non-local genotypes into surrounding bushland fragments (Bischoff et al., 2010).
Consequently, my objective is to evaluate the management of genetic diversity and reproductive output of *Banksia attenuata* within a restoration site established with low plant species diversity. This evaluation was achieved by contrasting these parameters for this species within a restored site established with high species diversity, and to remnant stands adjacent to these two restored sites. In this way, I assessed the impact of initial species diversity in restored sites on the reproductive functionality, and future genetic diversity, of a keystone species of *Banksia* woodland. Specifically, I use microsatellite markers to:

(i) Compare the levels of genetic diversity in *B. attenuata* between restored sites of low and high species diversity and their adjacent natural sites;

(ii) Contrast the mating systems and reproductive output of *B. attenuata* in low and high complexity restored sites and their adjacent natural sites;

(iii) Identify the importance of adjacent natural sites as sources of trans-boundary pollinator services to restored sites through an assignment of paternity to offspring of *B. attenuata*, and pollen dispersal within and among restored and natural adjacent sites.

**METHODS**

**Study species**

*Banksia attenuata*, commonly known as the candlestick banksia or slender banksia, is a widespread tree or woody shrub species, growing on deep sand in woodlands distributed across the Swan Coastal Plain of Western Australia (George & Gardner, 1981; Corrick & Fuhrer, 2002; Bussell et al., 2006). *Banksia attenuata* is a keystone species of the Swan Coastal Plain used extensively in ecological restoration of sand quarries on the (Rokich et al., 2002). It *Banksia attenuata* is a completely a outcrossing species (Scott, 1980), which relies on numerous vertebrate and invertebrate pollinators services (Collins & Rebelo, 1987; He et al., 2009) for successful pollination and it is vital that its plant-pollinator interactions are restored for ecosystem functioning (Broadhurst & Young, 2007). Seeds are predominantly gravity-dispersed post-fire, landing in close proximity to the parent plant, with germinants occurring beneath the tree canopy.
Chapter 6: Restoration complexity

(Crosti, 2011). Seeds must germinate within the first winter, or they perish in the soil seed bank (He et al., 2009).

Study sites

This study was conducted at two sites containing *Banksia attenuata* located in Jandakot (32°06′28″S, 115°52′01″E), on the Bassendean sand belt, 21 kilometres southeast of Perth in Western Australia. The first site (with approximately 120 mature trees) is located within a post-mine restoration site (referred to as “Jandakot restored”) and the second site (with approximately 500 mature trees) is located within naturally occurring *Banksia* woodland adjacent to the restored site (referred to as “Jandakot natural”; Fig. 6.1). The two sites are in close geographical proximity (circa 150 m). The restored site is located within a 57-hectare post-sand-extraction site previously owned by Rocla Quarry Products leasehold. The *B. attenuata* plants in the restored population were 16 years old in 2011. Restoration was completed in 1995 and the *B. attenuata* plants reached reproductive maturity in 2007. Plants within the natural site were mature individuals likely to be up to 300 years old, surviving in the landscape since the pre-European period (Enright & Lamont, 1992; Lamont et al., 2007).

The initial planning for restoration efforts at Rocla’s Jandakot site satisfied completion criteria of the time. Topsoil generated from earlier stripping programs was spread over the site (sandy topsoil with limited organic matter approximately 0-150mm depth), conceivably increasing the amount of native species and a selection of species were chosen for green stock plantings. Dry summers and predation reduced survival rates considerably. The site was not supplemented with additional plantings or provided more substantial restoration, as there was uncertainty over future land use to the Final Landform Plan of 1995 (Rocla Quarry Products 1995), the objectives outlined an uncertain future for the restoration program due to the longer-term outlook of changed land use for the site i.e. residential or industrial use. This restored site will be referred to as a “low complexity” restoration site.

Results of this current genetic assessment at Jandakot were compared to a similar earlier assessment of restoration success within an award winning Rocla Quarry Products post-quarry site (Ritchie & Krauss, 2012). The study was conducted on two sites located at Gnangara (31°47′09″S, 115°56′32″E), 40 kilometres north-northeast of Perth in Western
Australia. One site was located within a restored site (referred to as “Gnangara restored”) planted in 1996, and the other within naturally occurring established Banksia woodland located adjacent to the restored site (referred to as “Gnangara natural”; Fig. 6.2). This restored site was reinstated with over 70% of woodland species, the highest achieved in a woodland community (Petroleum, 2011) and will be referred to as a “high complexity” restoration site.

Site species diversity

To compare the quality of plant species restoration in Gnangara and Jandakot, species presence data for the Jandakot restored site was obtained through the Final Landform Plan 1995 and personal observations (Appendix 3). The Jandakot Regional Park - Bush Forever Site 389 was chosen as a suitable reference site with eleven 10 x 10m quadrats surveyed (Clarke & Langley, 2000). The Gnangara sites were surveyed in Spring 2012 (RPS Consultants) using 20 x 20 m quadrats, 20 in the natural reference site and 51 in restored site (Gaskell Avenue). The survey efforts differed between Jandakot and Gnangara sites, so number of species was selected to quantify restoration complexity rather than abundance.

Flowering and fruiting; Assessment of reproductive output

Reproductive output was measured at the four sites at the commencement of this study. Ten mature \( B. \text{ attenuata} \) trees per site were tagged and monitored for seed production throughout the study (2011-2013). I recorded new inflorescences produced and the number of cones that bore follicles (infructescence) for each of the ten trees per site. Within the first year of the study, cones were collected from Jandakot restored and Jandakot natural sites. These ten monitored trees were also sampled for genetic analysis (see below).

Genetic sampling

Leaf samples of \( B. \text{ attenuata} \) trees from Jandakot were collected in 2011 representing 103 trees in the natural site and 99 trees in the restored site. The aim was to collect from all trees (approximately 100) within a given area (approximately 200 m\(^2\)) within each site. Of these, ten maternal trees were sampled from each site that bore enough seed-bearing cones to collect a minimum of 10 seeds from each tree. Four young leaves were collected from each tree and stored in labelled zip lock bags with silica gel crystals,
until DNA extraction. The exact location of each sampled tree was determined by Global Positioning System (GPS), enabling construction of a spatial distribution map (Fig. 6.1) and estimates of measures of within site spatial genetic structure. A total of 10 seed from each maternal tree were extracted by placing each cone into a hessian bag and soaked for 1 hour, then into an oven set at 150°C for 10-15 minutes until the follicles opened and seeds could be removed.

Figure 6.1 Location of Jandakot study site, southwest of Perth, Western Australia. Circles indicate *Banksia attenuata* trees; open circles indicate the location of sampled natural trees (green) and restored trees (orange); closed circles indicate sampled maternal trees in the natural (green) and restored (orange) site.
Figure 6.2 Location of Gnangara comparison site (Ritchie & Krauss, 2012) northwest of Perth, Western Australia. Circles indicate *Banksia attenuata* trees; open circles indicate the location of sampled natural trees (green) and restored trees (orange); closed circles indicate sampled maternal trees in the natural (green) and restored (orange) site.

**Microsatellite genotyping**

DNA was extracted from approximately 0.8 g silica-dried (about 4 leaves) using a CTAB (cetyltrimethyl ammonium bromide)-based procedure (as modified for extracting DNA from *Banksia hookeriana* (Doyle & Doyle, 1990; He et al., 2004). DNA was extracted from seeds using a Jobe’s extraction protocol (Jobes et al., 1995). Seed coats were separated from their radicles to avoid contamination of seed DNA by maternal tissue. Radicles were pulverized (directly in the extraction buffer with one glass bead and 0.5 g acid washed sand in a 2 ml tube) within a tissue and cell homogenizer FastPrep®-24 instrument (MP Biomedicals, Inc., Solon, OH). The instrument was set for 24 samples of 2 ml tubes and ran for 20 seconds. DNA pellets were resuspended in 75 µL of 10 mM Tris and 1 mM EDTA, and 2 µL (of 5 µg DNA/100 µL dH2O.) of this mixture was used in each polymerase chain reaction (PCR). Microsatellite amplifications were performed for nine polymorphic markers; BaA3, BaA112, BaC3, Ba5, BaB1, BaB106, BaC8, BaC112 and BaD115 (Appendix 3) previously designed for *B. attenuata* (He et al. 2004; 2007). Amplifications were performed, with the following PCR conditions: 96°C for 2 min (1 cycle), followed by 30 cycles 94°C for 1 min, 52 to
54°C for 1 min (according to each primer pair annealing temperature; Appendix), 72°C for 1 min and final extension time of 72°C for 4 mins. PCR products were stored at 4°C, before being separated by capillary electrophoresis using a Beckman Coulter CEQ 8800 Genetic analysis system. An internal size standard (SS400) was added to each well loaded on the CEQ 8800. Alleles were scored using the CEQ 8800 Genetic Analysis System program.

**DATA ANALYSIS**

*Site species diversity*

The level of plant species diversity was assessed in both restored sites, Jandakot and Gnangara, to quantify and compare the quality of restoration. Restored sites were compared to reference sites using Beta diversity, with Sorenson’s Similarity Index ($\beta = \frac{2c}{S_1+S_2}$, where $c$ is the number of species in common, $S_1$ is the number of species at site 1, and $S_2$ is the number of species at site 2). The index is used for comparing species communities, where the index ranges from 0 (no species overlap) to 1 (complete overlap) (Sørensen, 1948).

Variation in reproductive output between Jandakot and Gnangara, based on factors of inflorescence counts, follicle production and follicle:inflorescence ratio measures, was assessed using repeated measures Analyses of Variance (ANOVA). To test differences in reproductive measures between the restored sites and between restored and their adjacent natural site, I used multiple paired $t$-tests (comparing population mean values of each factor; inflorescence counts, follicle production and follicle:inflorescence ratios). Simple linear regression was used to test for predictive relationships between inflorescence and follicle production in 2011 and 2012.

*Genetic analysis*

The program Micro-Checker (van Oosterhout et al. 2004) was used to ensure that the data from each locus matched the simple sequence repeats for the individual microsatellites, and outlier alleles and possible presence of null alleles were identified. Population genetic parameters were estimated for adult and offspring populations using the software program GenAlEx 6.5 (Peakall & Smouse, 2012); number of alleles ($N_a$),
effective number of alleles ($N_e$), number of private alleles ($Pr$), observed heterozygosity ($H_o$), expected heterozygosity ($H_e$), Shannon’s information index ($I$) were estimated for and offspring populations and fixation index ($F_{is}$) for adults using the software program GenAlEx 6.5 (Peakall & Smouse, 2012). $N_e$ is the number of equally frequent alleles it would take to achieve a given level of gene diversity. The average heterozygosity over all loci is a good estimate of the extent of genetic variability in the population. The same parameters were estimated as reported in Ritchie and Krauss (2012) to allow for direct comparison between Gnangara and Jandakot sites. The program FSTAT (Goudet, 2001) was used to estimate genetic measures of allelic diversity, allelic richness ($Ar$) (equivalent of $H_e$) that are corrected for samples of different sizes, allowing comparison among populations. For each measure of genetic diversity, its mean and 95% confidence intervals were calculated. Significant differences between parameter estimates were declared if their 95% confidence interval did not overlap. An analysis of molecular variance (AMOVA) and pair-wise population differentiation ($F_{ST}$) was performed to partition the total genetic variation into within and among natural and restored site components using Arlequin ver 3.4.1.2 (Excoffier et al., 2005).

Inheritance of loci were confirmed by verifying the presence of at least one of the maternal alleles in each offspring. The program MLTR version 3.4 was used to estimate mating system parameters (Ritland, 2002) for each site, including the maximum-likelihood single ($t_s$) and multilocus ($t_m$) estimates of outcrossing rates, bi-parental inbreeding ($t_s-t_m$), correlated paternity ($r_p$) and the effective number of pollen donors per family ($1/r_p$) was calculated (Smouse et al., 2001). MLTR was used to confirm no scoring errors were made. The genetic diversity estimates were bootstrapped 100 times by re-sampling loci within families and the 95% confidence interval was estimated.

**Paternity assignment**

Likelihood-based paternity analysis was conducted using the program CERVUS 2.0 (Marshall et al., 1998; Kalinowski et al., 2007). The likelihood ratio is defined as the likelihood of a paternity of a particular individual relative to the likelihood of paternity of a random individual. Paternity is assigned to a particular individual if the logarithm of combined likelihood ratios derived at each locus, is larger than the likelihood ratios of other individuals. The logarithm of combined likelihood ratio is termed the logarithm
of odds (LOD) score. The Delta statistic is defined as the difference in LOD score between the first and the second most-likely candidate parents. Simulations were used to determine the threshold Delta scores at a given confidence level. In this study, the simulation parameters for CERVUS to assign paternity to the most-likely individuals with a known level of statistical confidence were set as follows: 10,000 cycles of simulation, 204 candidate parents (all sampled reproductive adults from both restored and natural populations), 0.01 as the proportion of loci mistyped (genotyping error ratio), confidence levels of 95.0 and 80.0%. To assign an offspring to a pollen donor, a maximum of two mismatches between the offspring, mother tree and putative donor trio was used and the tree with the highest LOD value was accepted as the parent of the seedling if the difference between its LOD score and the second most-likely candidate’s LOD score was greater than the threshold Delta.

The distance between the maternal trees and the assigned pollen donor was used to estimate the minimum, maximum and average pollen dispersal distances. These distances were used to construct a dispersal curve to describe pollen movement within and between the Jandakot natural and restored (geographically adjacent) sites. The percentage of offspring that were assigned to a pollen sire within or between the sites was plotted as a function of inter-distance between maternal trees and most-likely pollen donors. All mapped trees with maternal tree locations and their assigned offspring pollen donor contributions were created.

**Pollen pool structure and connectivity**

Differentiation in maternal tree pollen pools (Φ_{ft}) and effective density (d) across populations were estimated with TwoGener analysis within GenAlEx 6.5 (Peakall & Smouse, 2012). Effective number of pollen donors per family (N_{ep}) was estimated by 1/2 Φ_{ft,ft} and the effective pollination neighbourhood area (A_{ep} = N_{ep}/d (ha)) in TwoGener. The overall molecular differentiation between pollen pools (Φ_{ft}), together with its variance, was calculated following Smouse et al. (2001). The required sampling for TwoGener is less rigorous, as the mean pollination distance and the effective neighbourhood size are estimated from the heterogeneity of alleles within the sampled families and uses plant density estimates (obtained and estimated for trees/ha within each site).
Spatial differentiation of pollen pools was then examined using Pollination Graphs (Dyer et al, 2012), based on the Population Graph (PG) approach detailed in Dyer and Nason (2004). PGs analyse pollen pool covariance rather than among-strata adult structure. This graph-theoretic approach describes the spatial distribution of genetic covariance among strata using conditional genetic covariance to define a network topology describing multilocus genetic connectivity. In the network that is defined, nodes represent maternally sampled pollen pools and connecting lines represent links of significant conditional covariance (see Dyer et al, 2012 for more details). The paternal contribution to each offspring genotype was used identify the genetic structure of sampled pollen pools. These were inferred from the male pollen multilocus haplotypes for each offspring produced from the TwoGener analysis. In cases where the maternal individual and offspring had the same heterozygote genotype, the paternal contribution was assigned probabilistically, as described in Smouse et al. (2001) (Dyer et al. 2012). The PG was then estimated and visualised using gstudio and popgraph (Dyer 2009; Dyer et al. 2012) in R statistical package (v 3.1.0, R Core Team 2014). A linear regression analysis was then conducted between Pollen Graph node size, representing the genetic variability of pollen haplotypes sampled by each maternal individual, and inflorescence and follicle production of the maternal tree to test for any relationship.

**Spatial genetic structure**

Spatial genetic structure (SGS) within each site was estimated by spatial autocorrelation analysis within GenAlEx 6.5 (Peakall & Smouse, 2012). Distance classes of 10m (20 in total) were used to determine whether there was any correlation between genetic and geographic distances of trees. This distance class was selected for direct comparison with the Gnangara site (Ritchie & Krauss, 2012). Spatial structure graphs (correlograms) were produced for natural and restored adult populations with calculated correlation coefficient $r$ and confidence limits, as generated by 999 random permutations of the data and bound by the 95% confidence interval about the null hypothesis of no spatial structure (Peakall & Smouse 2012).

Overall spatial genetic structure in each population was also assessed using the $Sp$ statistic to directly quantify the magnitude of spatial genetic structure among
populations (Vekemans & Hardy, 2004). The $S_p$ statistics were calculated using kinship coefficients determined for each locus ($S_p = -b(1-F_1)$, where $b$ is the regression slope of $F_{ij}$ on ln (distance) and $F_1$ is the mean $F_{ij}$ value between individuals belonging to the first distance interval that should include all pairs of neighbours (Ritland, 1996). Data for all loci were combined within each population. This was performed with 10 000 permutations using SPAGeDi 1.3 (Hardy & Vekemans, 2002). The inverse of $S_p$ was calculated as an indirect estimate of Wright’s neighbourhood size, $Nb (Nb = 1/S_p)$ for each population.

RESULTS

Site species diversity

Twenty-one plant species were chosen for the planting program in the 1995 restoration of Rocla’s post-mine at Jandakot (Appendix 3). There was little similarity between the Jandakot restored site and the Jandakot Regional Park reference site, with $\beta = 0.089$ (see Fig. 6.3; A, B). Of the species surveyed within Jandakot restored, 43% were non-local (i.e. beyond their natural distribution). In comparison, the Gnangara restored site (Gaskell Road) and its reference site had greater similarity, with $\beta = 0.62$ (Fig. 6.3; C, D), and all species were of local origin. The Banksia woodland restoration at Gnangara (Fig. 6.4) was much more species diverse, with 149 native species shared with the natural reference site and another 35 native species also recorded (RPS Consulting). The Jandakot restored site included the two dominant overstorey species, $B. attenuata$ and $B. menziesii$. However, understory structural complexity was lacking in comparison to the diverse structural components present within natural sites and the Gnangara restored site. Species present in the restored sites that were absent from the reference sites were investigated further, for historical natural distribution and range (see Appendix 3).

Reproductive output

Mean inflorescence production by $B. attenuata$ at Jandakot restored site was significantly lower ($t_{df=2} = 2.78, P = 0.04$) than its adjacent natural site Fig. 6.5). Mean follicle production was significantly lower in the Jandakot restored site than Gnangara restored site ($t_{df=2} = 2.92, P = 0.03$) but not significantly different from Jandakot natural. Inflorescence:follicle ratio was not significantly different among sites (Fig. 6.5). There was no significant correlation between inflorescence production and annual rainfall ($P >$
0.05). Populations of *B. attenuata* showed a weak linear relationship between year and inflorescence and follicle production, exhibiting the strongest relationship with number of inflorescences and explaining greater than 16% of the variation in follicle production for *B. attenuata* (*P* < 0.001; Fig. 6.6a).

Figure 6.3 Photographs illustrating structural complexity at each site; A, Jandakot restored; B, Jandakot natural; C, Gnangara restored; D, Gnangara natural. Taken by author.
Figure 6.4 Number of plant species present in reference and restored *Banksia* woodland sites.
Figure 6.5 Reproductive output per plant in Banksia attenuata populations (a) mean inflorescence, (b) follicle production and (c) follicle to inflorescence ratio. Annual rainfall for each site (a) obtained from BOM (www.bom.gov.au). Data were not collected at JN and JR in 2010. Standard error bars are shown.
Chapter 6: Restoration complexity

Figure 6.6 Relationship between inflorescences and follicle production in *Banksia attenuata* sites were measured in (a) 2011 and (b) 2012. Linear regression lines and the results of regression analyses are shown. n = number of plants measured.

**Genetic diversity**

Seven polymorphic microsatellite markers were used to genotype 103 adult trees and 99 offspring seeds from the Jandakot natural site and 99 adults and 100 offspring seeds from the Jandakot restored site. The genotypic data was used for a direct comparison with the Gnangara populations in Ritchie and Krauss (2012) (Table 6.1). 95% confidence intervals indicated that there was no significant difference in all the genetic parameters between the four populations of adult trees and offspring. On average, both Jandakot and Gnangara restored sites display similarly high levels of allelic diversity; mean number of alleles (Jandakot; 11.43 ± 2.34, Gnangara; 11.00 ± 1.83) and private alleles (Jandakot; 1.71 ± 0.84, Gnangara; 2.43 ± 0.65) in comparison to their adjacent natural site (Table 6.1). Mean allelic richness was greatest overall for Jandakot restored (11.05 ± 1.85).
Table 6.1 Genetic diversity parameters and fixation index (95% confidence intervals in parentheses) for adult and offspring populations from both natural and restored populations of Banksia attenuata in Jandakot and Gnangara (Ritchie & Krauss, 2012).

<table>
<thead>
<tr>
<th></th>
<th>Jandakot (Natural)</th>
<th>Jandakot (Restored)</th>
<th>Gnangara (Natural)</th>
<th>Gnangara (Restored)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>103</td>
<td>99</td>
<td>94</td>
<td>106</td>
</tr>
<tr>
<td>N_a</td>
<td>8.57 (6.92-10.22)</td>
<td>11.43 (6.84-16.01)</td>
<td>7.86 (6.23-9.48)</td>
<td>11.00 (7.42-14.58)</td>
</tr>
<tr>
<td>N_e</td>
<td>3.34 (2.50-4.19)</td>
<td>3.88 (2.56-5.19)</td>
<td>3.29 (1.86-4.72)</td>
<td>3.54 (1.86-5.21)</td>
</tr>
<tr>
<td>H_o</td>
<td>0.63 (0.48-0.77)</td>
<td>0.57 (0.50-0.65)</td>
<td>0.60 (0.42-0.77)</td>
<td>0.59 (0.40-0.77)</td>
</tr>
<tr>
<td>H_e</td>
<td>0.66 (0.55-0.77)</td>
<td>0.69 (0.60-0.79)</td>
<td>0.60 (0.44-0.75)</td>
<td>0.61 (0.44-0.78)</td>
</tr>
<tr>
<td>F_is</td>
<td>0.074</td>
<td>0.169</td>
<td>0.012</td>
<td>0.064</td>
</tr>
<tr>
<td></td>
<td>(-0.043-0.191)</td>
<td>(0.123-0.213)</td>
<td>(-0.072-0.097)</td>
<td>(-0.025-0.153)</td>
</tr>
<tr>
<td>I</td>
<td>1.43 (1.14-1.72)</td>
<td>1.45 (1.33-2.05)</td>
<td>1.29 (0.89-1.69)</td>
<td>1.40 (0.95-1.86)</td>
</tr>
<tr>
<td>Pr</td>
<td>0.43 (-0.15-1.01)</td>
<td>1.71 (0.02-2.98)</td>
<td>0.29 (-0.08-0.65)</td>
<td>2.43 (1.16-3.70)</td>
</tr>
<tr>
<td>Ar</td>
<td>8.33 (5.80-10.86)</td>
<td>11.05 (7.42-14.67)</td>
<td>7.03 (5.44-8.61)</td>
<td>8.76 (5.91-11.60)</td>
</tr>
<tr>
<td>Offspring</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>99</td>
<td>100</td>
<td>60</td>
<td>47</td>
</tr>
<tr>
<td>N_a</td>
<td>8.57 (5.55-11.59)</td>
<td>8.14 (6.46-9.82)</td>
<td>6.86 (5.07-8.64)</td>
<td>6.57 (4.92-8.22)</td>
</tr>
<tr>
<td>N_e</td>
<td>3.47 (2.56-4.84)</td>
<td>3.43 (2.03-4.84)</td>
<td>2.86 (1.98-3.73)</td>
<td>3.05 (1.64-4.46)</td>
</tr>
<tr>
<td>H_o</td>
<td>0.61 (0.53-0.69)</td>
<td>0.56 (0.43-0.68)</td>
<td>0.63 (0.44-0.80)</td>
<td>0.61 (0.42-0.81)</td>
</tr>
<tr>
<td>H_e</td>
<td>0.65 (0.54-0.76)</td>
<td>0.62 (0.44-0.77)</td>
<td>0.58 (0.41-0.74)</td>
<td>0.57 (0.39-0.73)</td>
</tr>
<tr>
<td>I</td>
<td>1.39 (1.03-1.76)</td>
<td>1.34 (0.99-1.71)</td>
<td>1.19 (0.87-1.51)</td>
<td>1.20 (0.81-1.59)</td>
</tr>
<tr>
<td>Pr</td>
<td>0.57 (0.18-0.97)</td>
<td>0.71 (0.15-1.27)</td>
<td>0.14 (-0.14-0.42)</td>
<td>0.0</td>
</tr>
<tr>
<td>Ar</td>
<td>8.28 (5.24-11.31)</td>
<td>7.96 (5.48-10.44)</td>
<td>6.47 (4.86-8.07)</td>
<td>6.70 (5.01-8.39)</td>
</tr>
</tbody>
</table>

N, Total number of samples; Na, average number of alleles per locus; Ne, effective number of alleles; H_o, average observed heterozygosity; H_e, average expected heterozygosity adjusted for sample size; F_is, fixation indices; I, Shannon’s Information Index; Pr, number of private alleles; Ar, mean allelic richness adjusted for sample size.
Figure 6.7 Allelic variation for natural and restored adult and offspring populations of Banksia attenuata at Jandakot. Na, Average number of alleles per locus; Ne, effective number of alleles; I, Shannon’s Information Index; Pr, number of private alleles and He, average expected heterozygosity adjusted for sample size.
Population differentiation

AMOVA partitioned 92.93% of the total variation within and 7.07% among the adults of the Jandakot natural and restored populations (Table 6.2) and 90.57% within and 9.43% among Jandakot offspring (Table 6.2). Adults and offspring demonstrated a low, but significantly greater than zero, degree of gene differentiation among populations in terms of allele frequencies ($F_{ST} = 0.071, P < 0.0001$ (adults) and $0.094, P < 0.0001$ (offspring)) also indicating weak genetic divergence among populations. Genetic divergence was larger than the reported 99% within and 1% among the adults and 96% within and 4% among offspring of the natural and restored populations in Gnangara (Ritchie & Krauss, 2012). Reported $F_{ST}$ was also weak in Gnangara populations, over 10 times lower in adults ($F_{ST} = 0.006, P = 0.01$ (adults) and $0.043, P = 0.01$ (offspring)) between Gnangara populations (Ritchie & Krauss, 2012).

Table 6.2 Analysis of molecular variance (AMOVA) for adult and offspring populations of *Banksia attenuata*. Variance was calculated between one restored population and one natural population in Jandakot, using 7 polymorphic microsatellite markers. Statistics include sums of squared deviations (SSD), variance component estimates, percentage of total variance (% total) contributed by each component, and the probability ($P$) of obtaining a more extreme component estimate by chance alone.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>SSD</th>
<th>Variance Component</th>
<th>% of Total</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jandakot Adults: Natural/Restored</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among populations</td>
<td>1</td>
<td>20.35</td>
<td>0.09</td>
<td>7.07</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Within populations</td>
<td>406</td>
<td>449.76</td>
<td>1.23</td>
<td>92.93</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>407</td>
<td>520.10</td>
<td>1.32</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Jandakot Offspring: Natural/Restored</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among populations</td>
<td>1</td>
<td>8.24</td>
<td>0.04</td>
<td>9.43</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Within populations</td>
<td>396</td>
<td>150.30</td>
<td>0.38</td>
<td>90.57</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>397</td>
<td>158.54</td>
<td>0.42</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>
Paternity assignment and realised pollen dispersal

The total exclusion probabilities over all eight loci for the first and second parents were 3.752 and 0.449, respectively. Paternity was assigned to 42 seeds (21%) with 95% confidence and 84 seeds (42%) with 80% confidence. Out of these, 41 out of 100 seeds from the Jandakot restored site were assigned paternity, and 43 out of 99 (43%) seeds from the Jandakot natural were assigned paternity. Of the restored site seeds that were assigned paternity, 22 (54%) were assigned to a pollen donor within the restored site and 19 seedlings (46%) were assigned paternity to a pollen donor from the natural site (Fig. 6.8). The shortest distance of realised pollen movement was 0 m (plus or minus 4 metre GPS error) the furthest was 377 m (Fig. 6.9). Of the natural site seedlings that were assigned paternity, 17 (40%) were assigned to a pollen donor within the natural site, and 26 (60%) were assigned to a pollen donor from the restored site (Fig. 6.8). The shortest pollen dispersal distance was 7 m and the furthest was 359 m (Fig. 6.9).

From these assignments, the average distance was 171 m (range 0-377 m) (Fig. 6.9), which mirrored the results of the Gnangara study (average was 141 m, range 2-324 m) (Ritchie & Krauss, 2012). Of the 43 seedlings in the Gnangara natural site, 51% were sired from nine trees (JN10, JN32, JN62, JN67, JN71, JN48 and JR58, JR102, JR104) of which four were maternal trees. Within the Gnangara restored site, 22% of offspring were sired from four restored trees (JR22, JR63, JR72 and JR91). Paternity/maternity of 14 seed (16%) was also shared (half-siblings) between both sites (JR22, JR63, JR115, JN67 and JN48) (Fig. 6.8).
Figure 6.8 Map of Banksia attenuata trees in the natural (top; squares) and restored (bottom; circles) sites at Jandakot, indicating the 10 mother trees from which seed was sampled. Filled squares and circles indicate trees that were genotyped. Each pie contains 10 genotyped seed collected in 2011, labeled with the mothers ID. Each segment indicates the source location of the pollen donor tree: natural (green), restored (orange), or unassigned (white). Joined segments indicate a shared pollen donor.
Figure 6.9 Distance class distributions of pollen flow inferred from parentage analysis of *Banksia attenuata* seeds sourced from Jandakot natural and restored sites. Paternity analysis based on relative LOD scores to potential fathers of 43 offspring sourced from the natural site and 41 offspring from the Jandakot restored site. Sires from the natural site are in green and sires from restored site are in orange bar graphs.
Pollen pool differentiation

Estimates of outcrossing were not significantly different from 1.0 at the Jandakot natural and restored sites, similar to estimates obtained for the two Gnangara sites (Table 6.3). Biparental inbreeding was low in all sites within Jandakot and Gnangara, although natural sites were slightly higher than restored sites (Table 6.3). Estimates for correlated paternity were nearly twice as high in the Jandakot sites than the Gnangara sites, although this was not significantly different. Correspondingly, both natural and restored sites in Jandakot had lower estimates of effective numbers of pollen donors per family when compared to the Gnangara site (Table 6.3).

Statistical differentiation among sampled pollen pools showed that populations of paternal pollen haplotypes sampled from mothers within both restored sites were more differentiated than those sampled within the adjacent natural sites ($\Phi_{st}^{\text{Restored}}$, Jandakot = 0.176, Gnangara = 0.170, $\Phi_{st}^{\text{Natural}}$, Jandakot = 0.118, Gnangara = 0.136) (Table 6.3). Pollination graphs produced for each population confirmed the MLTR, TwoGener pollen pool and Cervus paternity results displaying high connectivity among pollen pools between restored and adjacent sites at Jandakot. All families sampled were connected, with 6 out of 10 from the restored site connected to the natural site by 12 edges (Fig. 6.10), representing the links of significant conditional covariance.
Table 6.3 Mating system parameters (and their 95% confidence intervals shown in parentheses) for natural and restored sites of *Banksia attenuata*, estimated using open-pollinated offspring from 10 trees per site in Jandakot and 5 trees per site in Gnangara (data obtained from Ritchie & Krauss, 2012).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Jandakot Natural</th>
<th>Jandakot Restored</th>
<th>Gnangara Natural</th>
<th>Gnangara Restored</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLTR mating system</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multilocus outcrossing rate ($t_m$)</td>
<td>0.985</td>
<td>0.982</td>
<td>1.200</td>
<td>0.992</td>
</tr>
<tr>
<td></td>
<td>(0.926-1.044)</td>
<td>(0.929-1.035)</td>
<td>(1.198-1.202)</td>
<td>(0.901-1.083)</td>
</tr>
<tr>
<td>Singlelocus outcrossing rate ($t_s$)</td>
<td>0.822</td>
<td>0.884</td>
<td>1.095</td>
<td>1.074</td>
</tr>
<tr>
<td></td>
<td>(0.769-0.875)</td>
<td>(0.856-0.912)</td>
<td>(1.056-1.134)</td>
<td>(1.023-1.125)</td>
</tr>
<tr>
<td>Biparental inbreeding ($t_m - t_s$)</td>
<td>0.162</td>
<td>0.097</td>
<td>0.105</td>
<td>-0.082</td>
</tr>
<tr>
<td></td>
<td>(0.092-0.232)</td>
<td>(0.069-0.125)</td>
<td>(0.066-0.144)</td>
<td>(-0.132-0.032)</td>
</tr>
<tr>
<td>Correlated paternity ($r_p$)</td>
<td>0.108</td>
<td>0.101</td>
<td>0.074</td>
<td>0.075</td>
</tr>
<tr>
<td></td>
<td>(0.038-0.178)</td>
<td>(0.055-0.147)</td>
<td>(-0.143-0.291)</td>
<td>(-0.136-0.286)</td>
</tr>
<tr>
<td>Effective number of pollen donors ($1 / r_p$)</td>
<td>9.3</td>
<td>9.9</td>
<td>13.51</td>
<td>13.33</td>
</tr>
<tr>
<td>TwoGener analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Differentiation in pollen gene pool among families $\Phi_n$</td>
<td>0.118</td>
<td>0.176</td>
<td>0.136</td>
<td>0.170</td>
</tr>
<tr>
<td>Number of pollen donors $N_{np} = (1 / 2\Phi_n)$</td>
<td>4.2</td>
<td>2.8</td>
<td>3.7</td>
<td>2.9</td>
</tr>
</tbody>
</table>

Multilocus outcrossing rate ($t_m$), singlelocus outcrossing rate ($t_s$), bi-parental inbreeding rate ($t_m - t_s$), correlated paternity ($r_p$), and the inverse of correlated paternity as a measure of the effective number of pollen donors were estimated using MLTR 3.4 (Ritland 2002). Paternity differentiation between families ($\Phi_n$) was estimated based on AMOVA using TwoGener in GenAlEx 6.5 (Smouse et al. 2001; Peakall and Smouse 2006, 2012).
Figure 6.10 Conditional genetic covariance among pollen pools for Jandakot natural and restored sites; A, Pollination Graphs overlaid on a satellite image of the site and B, depicted in 3-dimensional space. Nodes represent the population of pollen haplotypes sampled by each maternal individual. Edges represent significant statistical covariance among connected pollen pools.
**Spatial genetic structure**

Spatial autocorrelation analysis identified significant \( r = 0.54; \ P = 0.005 \) genetic structure up to an interplant distance of 10 m within the Jandakot natural site (Fig. 6.11). The correlation coefficient was not significantly different from zero in the Jandakot restored site, indicating no genetic structure. The kinship coefficient \( (Sp \) statistic) was very low in both Jandakot sites (Jandakot; Natural, \( Sp = 0.002 \), Restored, \( Sp =0.001 \), Gnangara; Natural, \( Sp = 0.006 \), Restored, \( Sp =0.002 \)). The ‘neighborhood’ \( (Nb) \) estimates for the Jandakot natural and Jandakot restored were greater than Gnangara sites, \( Nb = 476 \) and \( Nb = 819 \), respectively.

![Spatial autocorrelation analysis correlograms for Banksia attenuata at Jandakot Natural (green) and Jandakot Restored (orange) showing the genetic correlation coefficient \( (r) \) for increasing distance class sizes, with 95% confidence intervals about \( r \) as determined by bootstrapping.](image-url)
DISCUSSION

The level of initial species diversity within a restoration site has little impact on key genetic parameters of reproductive functionality in *Banksia attenuata*. Despite low species diversity in the restoration site, ecosystem processes of pollination and subsequent gene flow were established for *Banksia attenuata* to a level comparable to that of the high species diversity restoration site (Ritchie & Krauss, 2012). An examination of genetic diversity and structure within and among restored and natural sites and their offspring revealed no signs of reduced genetic variation or elevated inbreeding within the restored population. Genetic connectivity through extensive pollen dispersal occurred between restored and natural sites, indicating that plant-pollinator associations were restored. Successful population integration was accomplished with the use of genetically diverse propagules, likely sourced from multiple source populations and/or from a wider locality than the restored site locality. Production of inflorescences within the low quality restored site was sufficient to attract pollinators, and thus provide effective pollinator services, resulting in the production of genetically robust outbred seed.

This study confirmed that the genetic management of *B. attenuata* in the low diversity Jandakot restored site was comparable to that of the high diversity Gnangara restored site (Ritchie & Krauss, 2012). Although genetic differentiation ($F_{ST}$) between the restored and natural sites was higher for Jandakot, there was no significant indication of reduced heterozygosity or genetic bottlenealing. High allelic diversity and richness within the Jandakot restored adults verified the use of genetically diverse propagules in the establishment of the site, with a relatively high $F_{ST}$ between the restored and natural sites (Jandakot: 0.071 Gnangara: 0.006) suggesting plant material used at the restored site may have been sourced from multiple populations and/or a greater geographical range (i.e. not local). Most trees that survived to reproductive maturity at Jandakot were from direct greenstock plantings. Survival rates were much lower from the topsoil seedbank (personal communication with Rocla Southern Quarry Manager T. Taylor, 2010), likely contributing to genetic differentiation between the restored site and its adjacent natural site. Using a mixture of greenstock, seed and topsoil for vegetation establishment in *Banksia* woodland is common practice, and when local nursery stock is not supplied from local sources these associated issues may arise (Knapp & Rice, 1996; Kettle et al., 2008; Aavik et al., 2012). If successful transfer of topsoil from pre-mining
or local area can be achieved, and the soil seed bank can successfully germinate, this will decrease the probability of introducing potentially maladapted, non-local propagules (see Rokich & Dixon, 2007; Ritchie & Krauss, 2012).

Whilst the Jandakot restored population may not currently show any adverse effects of using non-local seed, their use may have implications for the future fitness of this population. For example, (Aavik et al., 2014) indicated that even though local plants did not outperform those from seed suppliers (non-local), reproductive output, such as flowering period, differed between local and non-local sources. The measures of inflorescence production were significantly lower in the Jandakot restored site than its adjacent natural site, and trees at Jandakot produced a lower amount of follicles than the high quality restored site at Gnangara. Although there was evidence of lower inflorescence and follicle production, genetically diverse outbred seed was produced at the restored Jandakot site. Production of sufficient qualities of seed is essential for population persistence in sexually reproducing plants for dispersal (Wilcock & Neiland 2000). However, in this long-lived species with naturally low levels of seed set, seedling recruitment and survival should be measured to determine ecological resilience and the long-term stability of the population (Monie et al., 2013).

Mating system parameters in the restored Jandakot site were similar to those of the natural site and those in the previous study of B. attenuata in the high complexity restored site (Ritchie & Krauss 2012). These findings are consistent with other Banksia species (Coates & Sokolowski, 1992; Coates et al., 2007; Krauss et al., 2009; Frick et al. in press), indicating the successful restoration of pollinator services, despite the overall reduction in vertebrate pollinators (species diversity and abundance). Following habitat disturbance, vertebrate pollinator reduction can be compensated to some extent with increased invertebrate pollinators, as observed in the greater number of A. mellifera within the Jandakot restored population (Chapter 3). However, Celebrezze and Paton (2004) and England et al., (2001) showed that increased visitation by A. mellifera significantly reduced fruit quantity and/or seed production in comparison to vertebrate visitation. This may be a result of greater geitonogamy, resulting in the abortion or production of poor quality seed (England et al., 2001; Wooller & Woller, 2004; Heliyanto et al., 2005). The lower follicle;inflorescence ratio and effective number of pollen donors and higher correlated paternity in the Jandakot restored site are consistent
with such an explanation. Many other studies of bird pollinated species have recorded lower successful pollination events (England et al., 2001; Wooller & Woller, 2004; Hargreaves et al., 2010), and some label *A. mellifera* as pollen robbers (Goulson, 2003). *Banksia attenuata* is a species resilient to the genetic consequences of pollinator impacts, as it is preferentially outcrossing and relies on mixed insect and avian generalist pollinators. Consequently, the species reproductive biology counteracts pollinator behavior, which may promote selfing (geitonogamy) (Carthew et al., 1996; Goldingay & Carthew, 1998; Broadhurst & Young, 2007) through intra-tree foraging bouts (Chapter 4).

The re-establishment of pollination services is a critical component of successful restoration for reproductive potential and genetic resilience of restored populations of plants (Dixon 2009; Menz et al. 2012). Analyses of pollen-mediated gene flow in *Banksia attenuata* using direct (paternity assignment) and indirect (pollen pool covariance) measures showed that there is extensive connectivity between low quality restored and natural adjacent sites at Jandakot. Paternity assignment analyses in *B. attenuata* demonstrated effective pollen dispersal between two restored sites with their respective neighbouring natural populations. This study confirmed the delivery of pollinator services for *B. attenuata* in restored sites was possible with the close proximity to quality natural bushland fragments (see Chapter 3). Paternity assignment and realised pollen flow distances revealed natural and restored offspring have multiple pollen donors, with pollen donors for 60% of the natural offspring assigned paternity coming from within the restored site. These results were further verified by the numerous significant links of conditional covariance between and among pollen pools in restored and natural sites.

Allelic richness decreased in the Jandakot restored offspring, whereas, it was increased within the offspring of the adjacent natural site. These results indicate that the introduction of novel genetic material from the restored population into the adjacent natural population. For outcrossing species that may be more locally adapted within native local populations, the introduction of non-local genetic material through restoration may be detrimental, leading to outbreeding depression (Hufford & Mazer, 2003) (McKay et al., 2005; Carter & Blair, 2013). Few studies have investigated the trade-off between the potential benefits of genetic rescue or the negative outcomes from
outbreeding depression (Broadhurst & Young, 2007; Marsden et al., 2013). Paschke et al. (2002) concluded that increased available pollen donors led to greater seed production, outweighing any harmful consequences of outbreeding depression on the fitness of resulting offspring. This may be because the harmful effects of outbreeding depression may take many generations to appear. Experimental studies on *Banksia ilicifolia* (Heliyanto et al., 2006) and the aquatic *Vallisneria americana* (Marsden et al. (2013) using within and among-population crosses, resulted in fitness advantages from among-population crosses. These findings indicate the potential for heterosis in a variety of species, but that general practice guidelines applied in restoration should be species specific due to the different responses (e.g. *B. menziesii* higher inflorescences in Jandakot restored site, Chapter 3). This research confirms the importance of considering the source of plant material, when integration with local bush land remnants is possible, or desired.

Spatial genetic structure among populations, as measured by the $S_p$ statistic, has been applied to a diverse range of species (Vekemans & Hardy, 2004). Both restored and natural sites at Jandakot and Gnangara had very low $S_p$ values (range $S_p = 0.001$-$0.006$), but are comparable with studies in other populations of *B. attenuata*, *B. menziesii* and *B. hookeriana* where $S_p$ ranged from 0.002-0.010 (Krauss et al., 2009; Ritchie & Krauss, 2012) (Frick et al.; Chapter 5). These low numbers found in *Banksia* are an order of magnitude lower than the mean values provided for specific biological characteristics; outcrossing ($S_p = 0.0134$), trees ($S_p = 0.0102$), animal dispersed pollen ($S_p = 0.0171$), and gravity-dispersed seed ($S_p = 0.0281$) (Vekemans and Hardy (2004). They are, however, higher than *Virola michelii* a self-incompatible, animal-pollinated, animal-dispersed seed species, which has the lowest reported $S_p$ value (0.00031; Caron unpubl. in Vekemans & Hardy, 2004).

Low levels of spatial genetic structure (SGS) were not unexpected in *B. attenuata* despite gravity-dispersed seed, due to the slow-growing, long-lived nature of the species. Significant structure was, however, detected within natural sites at a 10 m distance class at Jandakot, and up to 30 m at the Gnangara site (Ritchie & Krauss, 2012). With the dispersal mechanisms employed by the species, the age of the population, declines in fecundity and decreased serotiny due changed fire regimes (Crosti, 2011; He et al., 2011), SGS breaks down (Cavers et al., 2005). Correspondingly
SGS was not detected in the Jandakot restored site, the methods used to establish restored sites, breakdowns any possible SGS. The mating between non-related near-neighbours would result in potentially successful pollination events and production of viable seed. Importantly, with reduced wild sources of seed for restoration projects, these findings show that the lack of structure suggests that seed collectors can collect from fewer trees within a smaller scale with little risk of negative genetic consequences.

Despite the Jandakot restored site containing only 5% of the native species found in the local natural bushland reference sites, this study has established that the establishment of reproductive functionality has been achieved in \textit{B. attenuata}. This was demonstrated through successful pollination events resulting in the creation of genetically robust seed. However, it is yet to be determined what the possible undesirable consequences may be as a result of poor community structure, low diversity, low density of understory native species, and the introduction of non-native species. For example, Geraldton wax, \textit{Chamelaucium uncinatum}, was planted into the restored site. It is not native to the local area and has the capability to become invasive, posing threats to nearby bushland by becoming an invasive ‘weedy’ species (Brown & Brooks, 2002). It is also very attractive to \textit{Apis mellifera} (European Honeybee) visitation. Lopezaraiza–Mikel et al. (2007) demonstrated that generalized native pollinators, including \textit{A. mellifera}, provided a pathway of integration for alien plants into native visitation systems. Heleno et al. (2010) demonstrated that with the removal of alien species within restoration sites, positive results were seen to flow through the food web from native plants to herbivorous insects, insect parasitoids and birds. The consequences of alien and reduced floral resources on pollinator limitation at the ecosystem-level have not been determined (see Bjerknes et al., 2007; Bartomeus et al., 2008). However, in comparison to Gnangara, the Gnangara restored site was of superior ecological quality (and did not contain any actively introduced non-native and non-local species).

Efforts targeted in restoring ecosystem functions, such as pollinator processes, must take into account the role that particular species may play in re-establishing these services (Palmer et al., 1997). \textit{Banksia attenuata} appears to be a good model species for investigating the restoration of pollinator processes in \textit{Banksia} woodlands. The dominant structural role of this species as a framework plant suggests it is a good candidate as a pioneer for restoring pollinators to a site (large attractive flowers). The
target endpoint in the majority of restoration projects is to have appropriate species rich communities that have restored functionality.

Though these findings are based on the comparison between two restored sites with lack of suitable replicates, I have demonstrated that the restored functionality of a keystone species, may in succession facilitate the delivery of pollinator services to other plant species within the restored community. Well planned local seed sourcing with a successful restoration outcome has been demonstrated in the previous *B. attenuata* (Ritchie & Krauss 2012) and *B. menziesii* study (Frick et al. in press) in Gnangara as well as internationally within prairie land restoration (Gustafson et al, 2005). The risk that non-local provenance seed were used for the low quality site has been shown to have no immediate negative effects for the population or its adjacent natural site, however, genetic differentiation effects and level of local adaptation needs further investigation.

Despite having low plant species diversity, pollinators for *B. attenuata* were present in the restored Jandakot site. However, without the successful establishment of a framework species, it is unlikely that these generalist pollinators would be attracted to or sustained by the restored site. Identifying possible framework or interactor plant species within restoration projects is beneficial to the restoration of pollinator services. To encourage colonization of restored sites by a diverse guild of pollinators, flowering phenologies of species selected for restored sites should be investigated. Choice of plant species selection at the local scale (in a restoration site) can affect the diversity of pollinators within the landscape scale and how well that site is integrated into the landscape. Monitoring of genetic differentiation and possible genetic pollution of adjacent natural sites as well as other plant community species is advised, particularly non-native introduced species’ pollinator networks and research into facilitating more specialized plant-pollinator networks is recommended.
Chapter 7: General Discussion

Western Australia is unique on a global scale in the diversity of pollinators that effect pollination services of native plants. This is no more evident than in *Banksia* woodland communities on the Swan Coastal Plain. Here, birds dominate the pollination of keystone *Banksia* and understorey species, providing critical service for seed production. However, understanding the how, when and what of pollinators in ecological restoration represents a critical missing link in established restoration practice. This study provides the foundation science to fill the knowledge gap for the re-establishment of pollination into restored *Banksia* woodlands, including understanding the genetic consequences ensuing for dominant plant species in restored communities. This research is of intrinsic importance to a strategic resource industry, in terms of completion criteria impacting on approval to operate, as well as providing insights that are more broadly applicable to restoration across the resource sector in biodiversity rich ecosystems.

Many studies have focused on the initial establishment of restored populations, measuring genetic structure and differentiation of natural and nursery sources for revegetation propagules (Lesica & Allendorf, 1999; Montalvo & Ellstrand, 2001; Hufford & Mazer, 2003; Rice & Emery, 2003; Broadhurst et al., 2008a; Bischoff et al., 2010; Aavik et al., 2012; Marsden et al., 2013). Few have evaluated restoration success using ecological and genetic indicators (García-Robledo, 2010; Munro et al., 2011; Williams, 2011). This study is one of the first to assess the restoration of ecosystem functionality in a biologically diverse ecosystem. This study provides a solid ecological genetic analysis for a better understanding of the driving mechanisms behind mating systems, pollinator behavior and mutualisms, and how these are influenced by anthropogenic disruption. To ensure long-term sustainable restoration, key population processes must be reinstated.

Specifically, the most important findings of this study were:

- The low complexity restored site contained lower insect diversity than the high complexity restored site seasonal differences in insect diversity and abundance
were detected. Also, insect functional groups were different for continuous, fragmented and restored sites. These last two findings highlight the importance of evaluating restoration outcomes at multiple temporal and spatial scales (Chapter 3).

- Bird diversity differed between continuous, fragmented, and restored sites. Continuous natural sites contained the greatest diversity of nectarivorous and insectivorous bird species. European Honeybee (*Apis mellifera*) visitation was highest within fragmented sites. Restored sites had different bird and insect pollinator species composition to adjacent natural sites, but shared elements indicate that these neighbouring natural sites are delivering limited trans-boundary pollinator services (Chapter 3).

- Bird foraging behaviour differed among site types, with intraspecific and interspecific aggressive chases increasing within restored sites. Larger bodied Western Wattlebirds and aggressive New Holland Honeyeaters dominated higher nectar resources of *B. menziesii*. The territoriality of these species influenced foraging patterns within restored sites and resulted in increased intra-tree and near-neighbour pollinator movements (Chapter 3).

- Inflorescence production was greatest within *B. menziesii*, particularly in the low complexity restoration site. In contrast, this site had the lowest *B. attenuata* inflorescence production. This difference in resource availability between the two *Banksia* species and thus season, influenced bird pollinator attraction and foraging behaviour (Chapter 3).

- Realised pollen dispersal distances were extensive within and among sites of *B. menziesii*, with the exception of the low complexity restored site. Results suggest that greater *B. menziesii* flowering within the restored versus natural site has reduced realised dispersal among the sites (Chapter 4).

- Trees of *B. attenuata* in natural populations adjacent to restored sites were sires (pollen donors) for 46% of the assigned offspring of trees in the restored site and 60% of assigned offspring in the natural site were sired by trees from the restored site. Consequently, despite the low complexity of restored community structure, there was no reduction in the delivery of pollinator services and reproductive functionality, with extensive connectivity through pollen dispersal with the adjacent natural site (Chapter 6).
• Mating system analysis showed *B. menziesii* and *B. attenuata* were completely outcrossing in all restored and natural sites and had low estimates of bi-parental inbreeding and correlated paternity (Chapters 4 & 6). Low pollen pool structure and multiple paternity within families supports extensive gene flow through effective pollinator services within each site.

• All stands of *B. menziesii* displayed high levels of genetic diversity, with restored sites having the greatest diversity in comparison to natural sites (Chapter 5). *Banksia attenuata* also exhibited higher allelic richness in restored sites than adjacent natural sites (Chapter 6). These results indicate that restored sites were established with levels of genetic diversity greater than that of comparably sized natural sites, indicating genetic mixing of seed sources.

• Genetic differentiation between restored and adjacent natural sites was greater for the low species diversity restored site at Jandakot than the high species diversity restoration site at Gnangara for *B. menziesii* (Chapter 5) and for *B. attenuata* (Chapter 6). These results indicate differences in the extent of propagule sourcing (provenance) for the two restored sites.

• Both *Banksia* species displayed weak spatial genetic structure within all populations, as demonstrated by a low *Sp* statistic (*Sp* = < 0.007) and spatial autocorrelation analysis (Chapters 5 & 6). These results illustrate a naturally weak local spatial genetic structure, than is even further weakened with more or less random dispersal of seed for restoration sites.

These main findings are discussed in the remainder of this chapter with regard to their implications for restoration and conservation of this biodiversity hotspot. I will then address some limitations of this study and identify key areas of future research.
Targeting non-target species in restoration

“I predict that Australia will lose half of its terrestrial bird species in the next century”
Recher, H.F. 1999

Pollinators and other mobile species are generally not targeted within restoration projects (Dixon, 2009; Menz et al., 2011; Munro et al., 2011; Williams, 2011). Pollinators are of great importance to the long-term functioning of restored ecosystems (Handel, 1997), yet species are left to colonize on their own, expected when conditions become appropriate (Williams 2011). With decreased native woodland, food resources have diminished across the Swan Coastal Plain (SCP), with many ecological implications for nectar feeding birds. There was considerable interest in the repercussions that decreased nectar resources had for honeyeater population densities within Australia in the 1990s (Armstrong, 1991b; Saunders, 1993), but little research has been done since. Although pollinators known to be in decline globally (Potts et al., 2010), the focus of research has largely been within northern hemisphere invertebrate pollination systems (Winfree et al., 2011). For their economic value, pollination has been well recognized and researched within agricultural and horticultural systems (Kremen et al., 2002; Klein et al., 2007). However, research on their re-establishment within native ecosystems is still limited (Dixon, 2009).

In the 1990s several studies researched bird abundance and diversity in the Southwest Australian Floristic Region (SWAFR) (Recher, 1999) and results indicated “nearly all” insect eating and nectar-feeding birds had declined since European settlement as a result of habitat loss (How & Dell, 1993). It does need to be noted that some species have benefited from European settlement and increased in numbers and distribution, for example scavenging birds; Australian Ravens, however these species have detrimental impacts on species such as honeyeaters. Despite this alarming report, little follow-up research has been done. So assuming this trend continued, significant components of the avifauna will have been lost through population extinction and from local to continental scales the composition of avifauna will have changed. While these changes may not necessarily be threatening to the pollinators and species concerned in this study, there has been noted disruption of species as a whole (Recher, 1999). Population declines of these animals may be due to exotic species such as cats and fox predation, competition for nesting sites by non-local species or food resources and by *Apis mellifera* (Paton,
This present study revealed changed pollinator communities across sites, but as yet, there are no lists of threatened pollinators within this region. Despite the lack of empirical data indicating decreases in abundance or diversity at a landscape-scale within the SCP, this study, along with other international research (Steffan-Dewenter et al., 2002; Melles et al., 2003; Pauw & Louw, 2012) indicates that largely, as habitat and vegetation complexity decreases, native bird and insect species decline in diversity and composition (See Fig. 7.1; O'Connell et al., 2000).

Figure 7.1 Forested sites in good and excellent condition supported diverse bird communities. In general, the downward shift from medium to poor ecological condition as defined by bird communities coincided with a shift in land cover composition from forested to non-forested in the Appalachians, Eastern United States of America. Taken from (O'Connell et al., 2000)

In the current study, the highest diversity of native birds was within natural continuous sites. Both high and low complexity restoration sites had similar pollinator composition and lower species diversity than in their respective adjacent natural sites (Chapter 3). Natural fragments in this study contained nesting sites of Western Wattlebirds and Western Spinebills contributing to pollinator conservation. These observations are consistent with several other studies (Estades & Temple, 1999; Comer & Wooller, 2002; Lindenmayer et al., 2002b; Jansen, 2005), suggesting that both local and landscape-scale resources are significant in determining the distribution of birds. Absence of these species indicates restored sites lack provisions to support or attract the
full range of species found in surrounding natural vegetation. These findings stress the importance of natural sites for conservation of pollinator diversity.

Re-establishing habitat characteristics that encourage the colonization of species in restored areas are mutualistic for both plants and their pollinators. Restoration projects within this biodiversity hotspot should amend their objectives to list the restoration of habitat characteristics for the recovery of native bird and insect communities. As a part of the recovery process, restoration needs to emphasize the use of habitat elements within natural communities such as tree logs, as well as using native indigenous vegetation across all structural components (i.e. life form diversity; trees, shrubs, grasses). These characteristics may take time to restore, although some of these can be added. Restoring function to the land requires more than planting trees, although this is an obvious and essential part of the process.

Theoretical studies have dominated research in this field, using conceptual frameworks for evaluating restoration success by modeling the trajectory of vegetation structure and species mutualistic interactions (e.g. (Hobbs & Norton, 1996; McIntyre & Hobbs, 1999; Hobbs & Harris, 2001; Amarasekare, 2004; Suding & Hobbs, 2009). Previous studies such as Amarasekare (2004), applied information such as the general mobility of pollinators into models to assess how plant density and spatial arrangement of a site will influence dispersal and connectivity, but not their behaviour or intraspecific and interspecific interactions. This empirical study of several Banksia woodland sites demonstrated that plant-pollinator interactions go beyond the general mobility of all the species, and the foraging behavior and interactions between these pollinators may be more of a determining factor on dispersal and connectivity. Identifying patterns can be useful to guide restoration and conservation planning, but assumptions that all species will confirm to the same patterns (e.g. (Kotliar & Wiens, 1990; Haines-Young & Chopping, 1996; Miller et al., 1997) may be too simplistic and informed detailed empirical studies such as this are needed.

From a genetic and ecological perspective, the two studied restored sites are connected to their adjacent natural sites in an urban matrix landscape, however the level of connectivity differed between site and between Banksia species. This study has shown that pollinator species diversity and behavior differs between landscape types and there
are differing characteristics such as vegetation structure and flowering intensity within restored sites that are limiting the movement of pollinators within and among these sites more than the physical distance between them. Knowledge of these interactions will help to define targets to achieve ecological restoration.

Government agencies responsible for land management generally concentrate on the reserves directly under their control, ignoring the remainder of the landscape, and organisations responsible for restoration projects generally have the same approach. Restoration of pollinators requires a broader landscape approach as these pollinators use the entire landscape, moving over man-made boundaries. Villard and Metzger (2014) reviewed the importance of habitat configuration in connecting fragmented and restored sites, explaining that habitat configuration, or landscape structure has the potential to reduce effects of habitat loss through inter-patch connectivity and maintaining functionality (Fig 7.2). Restoration within this urban matrix needs to be integrated into landscape to maintain resident avifauna and overall species diversity in this urban environment. Collaboration between ecologists, policy scientists, urban planner and local councils is needed for future successful management within the Swan Coastal Plain.

Figure 7.2 Spatial arrangement of natural habitat patches (shaded), and insertion of small restored habitat patch (black) in two different scenarios of (a) and (b). Different locations can result in very different effects on functional connectivity and the corresponding habitat network. Adapted from Villard and Metzger (2014).
New approaches to seed sourcing

Achieving successful landscape-scale restoration will require effective broadcast seeding. Where topsoil is not available, broadcast seeding is the most cost-effective means of restoring native vegetation to large areas (Merritt & Dixon, 2011). However we are facing a crisis of supply, as the current demand for seed is barely being met by wild sources (Suding et al., 2004; Broadhurst et al., 2006; Broadhurst et al., 2008a). A range of scalable, proven and cost effective methods such as seed farming, will be necessary to meet these demands (Merritt & Dixon, 2011).

Seed farming enterprises are proposed in response to the demand for native seed, addressing these shortfalls (Merritt & Dixon, 2011). Seed generally is a low-cost item ($B.\ attenuata \sim \$0.55 \text{ per seed; } B.\ menziesii \sim \$0.65 \text{ per seed, roughly equating to } \$600/\text{ha} \text{ per species; (Grose, 2013)}), compared to other establishment and management activities for restoration, such as the costs of planting (Mortlock, 2000) or moving topsoil ($22,000/\text{ha}, (Maher, 2009)). However, the cost of seed is rising with decreasing available native resources. In 2009, establishing post-mined Banksia woodland cost $16,500/\text{ha} (Maher, 2009) and the large volumes of seed required (tons) is becoming increasingly difficult to obtain (Hobbs & Cramer, 2008; Merritt & Dixon, 2011). Large-scale farming can decrease the pressure on wild sources but caution is needed. For example, careful consideration must be given to the genetic fitness and provenance of the selection of initial seed orchard material (Knapp & Rice, 1994; Lesica & Allendorf, 1999; Hufford & Mazer, 2003) (Fig. 7.3).
Figure 7.3 Reproductive functionality - the next link in the chain for successful ecological restoration. Adapted from Merritt and Dixon (2011).

Seed farming operations, to date, have focused on wind pollinated native grasses (Lodge, 2002; Cole & Johnston, 2006; Tischew et al., 2010) that have short generation cycles (annual), and in general can be grown in the absence of pollinators, relying on wind pollination or self-pollination (Knapp & Rice, 1994; Knapp & Rice, 1996). Those that require pollinators are generally restricted to bee pollination (Moncur et al., 1995; Cane, 2008). Recently there have been developments in applying these techniques to long-lived woody species internationally (Savolainen & Kärkkäinen, 1992; El-Kassaby et al., 2000; Vander Mijnsbrugge et al., 2005) and locally, for example, seed orcharding of Banksia species, a form of seed farming that occurs within a post-mined restoration area. One of the main limitations to the efficiency of this type of seed production is the low seed set rates in banksias (Cowling et al., 1987). The issue of how to gain the highest seed yield, until now, has received little attention. A new issue requiring consideration will be the generation of the quantities of seed to deliver the many tonnes likely to be required for restoration efforts (Merritt & Dixon, 2011).

Understanding the relationship between establishment of Banksia trees, other plant species within the Banksia woodland community, and their interactions with the required pollinator community can help address this need. Establishing plant-pollinator interactions and attracting the greatest diversity of pollinators (as level of pollination increases with diversity of pollinators; Albrecht et al., 2007) is of great importance, maximizing the potential seed output of orchards (Moncur et al., 1995). The findings in this study can be applied to improve the floral resource architecture of these future
orchards. As measured in Chapter 4, the low species diversity restoration site had higher \textit{B. menziesii} inflorescence production and follicle production, however, the percentage of viable seed was the lowest. This site structure reflects that of proposed seed orchards, with monoculture lines of keystone \textit{Banksia} species, \textit{B. attenuata} and \textit{B. menziesii}, with limited to no understory, and likely reticulated for improved establishment and survival. The findings in the study however, suggest that planting these species in isolation and targeting inflorescence production alone may not result in high seed yields. The clumping of these floral resource may result in territoriality of birds, increase aggressive chases, intra-tree and near neighbour foraging patterns, which may be more of a influencing force on seed production. Temporal changes need to be considered within these orchards, as well as position in the landscape. With the lack of overstorey and understory shrubs, the ambient temperature is hotter within these sites during summer, which in turn may reduce bird pollinator services, particularly if the site is isolated from natural populations.

The results of the current study suggest that buffer rows of other native species such as \textit{Adenanthos} (Proteaceae) that have overlapping flowering phenologies, are required to provide substantial nectar (Newland & Wooler, 1985) and protective habitat. These should be planted into proposed orchards, to maximize their attractiveness and deliver pollinator services to the banksias. For more practical implications generated from this work for the ecological restoration and re-establishment of functioning \textit{Banksia} woodland communities, see the recommendations in Box 7.1.

\begin{boxedminipage}{\textwidth}
\textbf{Box 7.1: Implications for the restoration and conservation of \textit{Banksia} woodlands}

\textit{The following guidelines address the goal of restoring and preserving the remaining landscape-scale connectivity, genetic diversity and structure of \textit{Banksia} woodlands. Recommendations are drawn from the results of the current study, and target restoration practitioners and governing bodies.}

\textbf{1. Distribution of floral nectar resources and vegetation structure support greater bird species, encouraging the reestablishment of diverse pollinators}

Observed differences in vegetation structure within and between restored and natural sites influenced pollinator visitation, behaviour and therefore foraging movements.
\end{boxedminipage}
Maintenance and establishment of natural vegetation in the shrub layer within restored sites increases the number of niches available for a larger number of bird species, particularly small-bodied honeyeaters. **Recommendation:** Disperse floral resources and understory species to decrease defensibility of larger bodied and aggressive honeyeater species, decreasing territoriality and facilitating smaller species to enter and forage on restored plants.

2. **Conserving continuous woodlands**
Large woodlands (>25 ha) are necessary to maintain high bird and insect species diversity and thus conserve greater ecosystem function. Anticipation of urbanization is needed with resourceful ways to increase native habitat and manage it collectively, reducing the effects of urbanization on once remote natural areas. **Recommendation:** Urban development restriction to grey-lands and disturbed lands, conserving and maintaining largely intact woodlands from development.

3. **Conserving fragmented remnants for conservation of key ecosystem functions.**
Maintaining native vegetation, deadwood, old trees and other nesting structures within bushland fragments are important for persistence of animal species. **Recommendation:** Public access and disturbance should be limited within these fragments. For example, walk trails, dumping of rubbish, arson should be kept to a minimum to conserve biodiversity within these small urban fragments.

4. **Conservation of adjacent natural sites benefits restored sites**
The observed pollinator movements and extensive genetic connectivity through pollen dispersal among restored and adjacent natural sites emphasizes that these natural sites deliver trans-boundary pollinator services to restored *Banksia* populations. **Recommendation:** Maintain implemented buffer zones and adjacent habitat throughout the restoration process.

5. **Maintaining and restoring corridors for delivery of pollinator services.**
Provide statutory recognition for the value of fragmented native habitat. The size of the habitat does not reflect the effective size of the population, as mating occurs in the
Knowledge gaps and future research requirements

The Southwest of Australia is a very old weathered landscape with an extremely diverse flora (Hopper & Gioia, 2004). The region has high degree of heterogeneity in the vegetation with an extremely patchy distribution of vegetation types (Hopper & Gioia, 2004). The distribution and abundance of the native biota is still largely unknown, and information on the basic biology and functional requirements of all but a few species is lacking (Box 2) (Saunders, 1993). Taxonomic and biological knowledge of invertebrates is extremely limited. For example, three species of flower visiting wasps (Thynnidae: Thynninae) caught in this study have not been taxonomically named (Brown, 2014). Even though many studies have collected biodiversity inventories in which species and species compositions have been identified, we still lack data on their biological traits and function (Saunders, 1993; Williams, 2011). For management of natural and restored sites to be successful, we need to know how bird and insect species are distributed, the numbers in which they still occur, how they move within and between fragments and what linkages are required to promote their movements between sites (Morrison, 2009) (Box 7.2).
Although bird pollinator presence, abundance and behaviour were recorded for the restored sites, no observations were made on whether these birds were resident and breeding within these sites. Successfully restored sites should contribute to pollinators being able to reproduce at a rate that allows their population to replace itself, the mere presence of individuals in a site is not strong evidence that the site contributes positively to individual reproductive success (Bock & Jones, 2004; Lindell, 2008). For example, measuring nest success and nest density of bird (Larison et al., 2001; Fletcher Jr & Koford, 2002) and bee species (Potts et al., 2005; Winfree, 2010) within the restored sites. Local communities, and practitioners should carry out inventories of the biota not only noting species presence but how they use the site, such as for food resources or nesting. Identification of species that are vulnerable, those that are dependent entirely on native vegetation for their survival and which have undergone a decline in range and/or abundance from urban expansion is also required. This will involve more research at the species, community and landscape level (Saunders, 1993).

In order to maintain the movements of wildlife corridors on a local scale, plantings of nectar resources in gardens and private residences can help facilitate the connections between fragments of natural vegetation (Parsons et al., 2003; Pauw & Louw, 2012; Davis & Wilcox, 2013). Planting native species into gardens and streets, specifically species with higher nectar resources can support pollinators when natural floral resources are low within native sites and assist the movement of species through the fragmented urban landscape (Parsons et al., 2003; Pauw & Louw, 2012) and into. Increasing native vegetation within the urban landscape will not only improve connectivity, but also may reduce ambient temperatures within urban settlements, which may facilitate greater dispersal distances of native insects (Gaston et al., 2010; Gilman et al., 2010; Nardoni Laws & Belovsky, 2010; Tomlinson et al., 2014).

It is evident that we do not have the time to wait for these results as ecological restoration and habitat loss within an already highly fragmented matrix are happening now. As a result, this study was limited in its experimental design. For example, at the commencement of this study, there was a large natural remnant adjacent to the Alexander Park study site. By the end of the study, it was cleared and subdivided for housing (Fig. 7.4).
Figure 7.4 Native *Banksia* woodland is being rapidly clearing for housing on the Swan Coastal Plain. Photographs taken by author.

**Box 7.2: Future research issues addressing reproductive functionality in restored populations of banksias**

1. **Understand the functional types colonizing restored sites**

There is still a large gap in knowledge of understanding the functional roles of species that first colonize a restored site, and those that do not, especially for insects. We lack taxonomical and biological knowledge of many species in the SWAFR, and there should be an attempt to consider fauna at the species level rather than the ordinal level within restoration research, with a move to have taxonomist fees built into research budgets. This research will lead to improved targets of restoration, as we can identify species complexity in natural systems and aim to develop it within restoration.

2. **Assess fitness consequences of wide outcrossing within offspring of adjacent natural sites**

This study revealed elevated allelic richness within offspring of an adjacent natural site, as a result of introgression of non-local genotypes from a genetically differentiated restored site. Further study is necessary to investigate the outcomes of this genetic mixing, to determine if heterosis or outbreeding depression may eventuate. Neutral genetic markers do not respond to selection as adaptive traits do (McKay & Latta, 2002). Advanced molecular techniques such as next generation sequencing, population
genomics or combined analysis of neutral molecular markers and quantitative traits can help make more decisive decisions in source material selection for restoration and test the effect of potential non-local restoration genotypes entering natural adjacent sites (Williams et al., 2014).

3. **Quantify the variation in flowering and annual nectar resources in restored sites.**

Post-mine sites have altered geology and landscape structure. Plant species restored at these sites can have altered physiology and flowering phenologies. In addition, climate change may lead to altered asynchrony between pollinator life cycles and food resources. These potential shifts in seasonality between restored and natural sites may effect nectar-dependent species movements and therefore the reproductive functionality of Banksia species. Restoration programs may need to investigate and implement strategies for pollinator facilitation, for example identifying and planting bridging species (that provide resources over resource-limited times) or magnet species (co-flowering species that have attractive flowers). This has been proved to improve agricultural environments, enhancing pollinator migration and colonization, and may lead to better restoration outcomes and/or seed orcharding, by increasing potential reproductive output.

4. **Investigate aggression behaviour**

Measuring restoration success through evaluating animal behavior could be a strategic and cost effective use of resources, identifying and measuring the behavior of a few key species that play significant ecological roles. Behavioural patterns often reflect habitat quality, and this study indicates that even short-term behavioural decisions, such as movement within a stand, reflect the quality of a pollinator’s habitat. These movements can also affect the potential genetic fitness of the plants, through pollen flow. Restoration ecologists can take advantage of these links by quantifying behaviour to assess the relative quality of restoration sites and to identify how honeypetter aggression may affect reproductive success for seed orcharding.

5. **To what extent are fragmented sites used as corridors.**

Research is critically needed to understand the mechanisms and permeability to the
movements of certain taxa between these fragments, as well as to identify drivers of habitat degradation i.e. do fragmented sites facilitate the functioning of metapopulations? Knowing how to create and maintain corridors that support movement of species rather than trap them is needed. Investigating species thresholds, the arrangement of flowering plants, annual nectar resources, scattered and standing dead trees etcetera, in these remaining woodlands will determine if they are functioning as ecological traps, benefiting conservation management and informing restoration ecologists. Increasing the potential mobility of pollinators increases the genetic connectivity of these fragmented habitats, and in turn decreases potential negative consequences of genetic isolation. This research will lead to better restoration outcomes through identifying facilities needed to increase facilitation and movements of animal species into restored sites, but also to encourage colonization.

6. Investigation into the effects of urbanisation on insect communities
Understanding how urbanisation effects insect communities can aid their conservation and restoration. As pollinators, seed dispersers and a food source for birds, especially during breeding seasons, however, very little is known about insect composition and abundance in urban areas. This study found that community composition of insects changed between site type, but there is little data on the correlation between variations in community composition and changes in functionality or viability of plant populations. Investigating how the spatial distribution of housing (and associated vegetation) affects insect population viability in surrounding fragments and may help future urban planning. We cannot reduce the amount of urban space, but we can control the design and its density.

7. Long-term investigations of animal establishment in restored areas.
Lasting partnerships with industry should allow long-term studies to be performed, which would provide more information within restoration, than that of generally chronosequence-type investigations. Comparison of sites of different age structures is not effective with the development of new technologies and improved restoration standards. Tracking the progression of a restored site will allow restoration ecologists to answer questions on animal establishment and the effects of restoration effort on
Concluding remarks

This study has confirmed that genetic diversity has been managed and reproductive functionality has been established within restored sites of *Banksia attenuata* and *B. menziesii*, despite marked differences in species diversity of the restored plant communities. Most restoration projects emphasise the early stages of recovery through site preparation to plant establishment, here I have demonstrated that integrating measures of ecological and genetic indicators at different temporal and spatial scales can be used to assess the success of restoration functionality within long-lived woody tree species. Both low and high complexity restoration sites received pollinator services at visitation rates equivalent to continuous natural sites, to support integrated populations that produced robust, outbred seed. Therefore, the attainment of the ‘functionality’ has been met, however decisions as to whether this level of functionality is accepted for restoration success, or whether adaptive management is necessary to achieve greater ecological restoration is still in question.

Avian and insect community biodiversity in the restoration sites assessed have not reached the levels represented in undisturbed habitat. Restored and fragmented sites were characterized by pollinator communities of lower species richness than those of adjacent and continuous natural sites. *Banksia* woodland has been recently and extensively reduced through urban expansion, and this rapid loss of natural vegetation emphasizes the requirement to achieve the highest possible level of functioning within restored *Banksia* woodlands. The immediate outcomes from altered pollinator behaviour are not detrimental to population genetic processes of these banksias, as they are obligate or preferentially outcrossing, have weak fine-scale spatial genetic structure, and have multiple, generalist, highly mobile bird pollinators. This demonstrates resilience to multiple restoration scenarios for these banksias, at least in the short term.
REFERENCES


References


References


References


Crosti, R. 2011. Recruitment of Banksia spp. in an anthropogenically disturbed mediterranean climate type woodland in Western Australia. Murdoch University.


Davidson, W. A. 1995. Hydrogeology and groundwater resources of the Perth region, Western Australia.


194


194


Goudet, J. 2001. FSTAT, version 2.9. 3, A program to estimate and test gene diversities and fixation indices. Lausanne University, Lausanne, Switzerland.


200
References


Keddy, P. A., and C. G. Drummond 1996. Ecological properties for the evaluation, management, and restoration of temperate deciduous forest ecosystems. Ecological Applications:748-762.


References


References


References


Shuey, J. 2013. Habitat Re-Creation (Ecological Restoration) as a Strategy for Conserving Insect Communities in Highly Fragmented Landscapes. Insects 4:761-780.


217


Williams, N. M., and R. Winfree 2013. Local habitat characteristics but not landscape urbanization drive pollinator visitation and native plant pollination in forest remnants. Biological Conservation **160**:10-18.


APPENDIX 1

Table 1 Numbers of hymenoptera and flower-visitors from their respective families captured by 36 Malaise Traps in each site 2010-2013.

| Group | Family/Order | Continuous | | Fragmented | | Natural | | Adjacent | | Restoration | |
|-------|--------------|------------|---|-----------|---|---------|---|------------|---|------------|
|       |              | Neaves North | Neaves South | Alexander | Highview | Marangaroo | Paloma | Gngara natural | Jandakot natural | Gngara restored | Jandakot restored |
| Bees  | Apoidea, Apidae, Colletidae | 27 | 10 | 5 | 4 | 21 | 14 | 17 | 30 | 21 | 20 |
|       | Megachilidae | 0 | 0 | 2 | 3 | 0 | 1 | 2 | 3 | 0 | 0 |
| Ants  | Formicidae | 27 | 26 | 22 | 17 | 12 | 5 | 18 | 3 | 25 | 122 |
| Wasps | Braconidae | 11 | 29 | 8 | 2 | 3 | 6 | 8 | 7 | 5 | 2 |
|       | Chalcidoidea | 2 | 0 | 1 | 0 | 0 | 3 | 4 | 1 | 2 | 0 |
|       | Chrysidioidea | 4 | 1 | 1 | 5 | 0 | 6 | 3 | 1 | 8 | 6 |
|       | Evaniidae | 73 | 58 | 22 | 48 | 30 | 49 | 85 | 3 | 50 | 9 |
|       | Gasteruptiidae | 2 | 2 | 0 | 0 | 1 | 1 | 1 | 1 | 2 | 0 |
|       | Ichneumonidae | 12 | 11 | 7 | 4 | 2 | 6 | 6 | 5 | 2 | 1 |
|       | Multitiidae | 3 | 3 | 2 | 0 | 1 | 0 | 0 | 0 | 2 | 1 |
|       | Pompilidae | 9 | 1 | 3 | 3 | 5 | 5 | 11 | 4 | 4 | 23 |
|       | Scolidae | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|       | Sphecidae | 5 | 2 | 4 | 4 | 2 | 20 | 2 | 0 | 0 | 0 |
|       | Thynninae | 1 | 4 | 1 | 4 | 2 | 9 | 10 | 3 | 3 | 0 |
|       | Tippiidae | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
|       | Vespoidea | 0 | 1 | 0 | 2 | 0 | 0 | 1 | 1 | 0 | 0 |
Table 1 continued…

<table>
<thead>
<tr>
<th>Group</th>
<th>Family/Order</th>
<th>Natural</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Continuous</td>
<td>Fragmented</td>
<td>Adjacent</td>
<td>Restored</td>
<td>Restored</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Neaves North</td>
<td>Neaves South</td>
<td>Gnegara</td>
<td>Jandakot</td>
<td>Gnegara</td>
<td>Jandakot</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alexander</td>
<td>Highview</td>
<td>Marangaroo</td>
<td>Paloma</td>
<td>natural</td>
<td>natural</td>
</tr>
<tr>
<td>Bee flies</td>
<td>Diptera: Bombyliidae</td>
<td>10</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Blow flies</td>
<td>Diptera:Calliphoridae</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Hover flies</td>
<td>Diptera: Syrphidae</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Muscoidea</td>
<td>Diptera: Anthomyiidae</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Flies</td>
<td>Diptera: Lonchaeidae</td>
<td>9</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Diptera: Nematocera</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Diptera: Tachinidae</td>
<td>2</td>
<td>6</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Diptera: Therioidea</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Diptera: Tipulidae</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Coleoptera</td>
<td>Cantharidae</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lepidoptera</td>
<td>Gelechiodea</td>
<td>23</td>
<td>12</td>
<td>7</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Geometridae</td>
<td>4</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total number of families</td>
<td>101</td>
<td>103</td>
<td>50</td>
<td>55</td>
<td>38</td>
<td>42</td>
<td>83</td>
</tr>
</tbody>
</table>
**Table 2** Diversity measures of observed pollinators from point counts 2010-2013 within each flowering season of *B. attenuata* and *B. menziesii* and within each site.

<table>
<thead>
<tr>
<th></th>
<th>Natural</th>
<th></th>
<th>Natural</th>
<th></th>
<th>Natural</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Continuous</td>
<td></td>
<td>Fragmented</td>
<td></td>
<td>Adjacent</td>
</tr>
<tr>
<td></td>
<td>NN</td>
<td>NS</td>
<td>±</td>
<td>AP</td>
<td>HP</td>
<td>MC</td>
</tr>
<tr>
<td>Number of Species</td>
<td>S</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Shannon Index</td>
<td>$H'$</td>
<td>1.28</td>
<td>0.97</td>
<td>1.12</td>
<td>1.05</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.16</td>
<td></td>
<td>±0.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simpson’s Index</td>
<td>$\lambda'$</td>
<td>0.67</td>
<td>0.51</td>
<td>0.59</td>
<td>0.65</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.08</td>
<td></td>
<td>±0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effective number of species</td>
<td>$H_{SR}$</td>
<td>3.60</td>
<td>2.64</td>
<td>2.87</td>
<td>2.10</td>
<td>2.70</td>
</tr>
</tbody>
</table>
## APPENDIX 2

### Table 1 Characteristics for 8 polymorphic microsatellite loci isolated from *Banksia menziesii*

<table>
<thead>
<tr>
<th>Locus</th>
<th>Repeat Motif</th>
<th>Primer Sequences 5’-3’</th>
<th>Dye Label</th>
<th>Anneal temp</th>
<th>Mg conc.</th>
<th>Size range (bp)</th>
<th>No. Alleles</th>
<th>Genbank Accession No.</th>
</tr>
</thead>
</table>
| BmA1    | (AC)18        | F: GCTGGAGATGGGATTTCTAG  
R: TGCTGAACCATGTTCCCTAT | D3        | 180         | 57        | 2.0             |            |                     |
| BmB6    | (CT)17        | F: CTCGCCCTCTATTTGGTG    
R: GGTTGCTAGTGAGGATGG  | D4        | 259         | 55        | 2.0             |            |                     |
| BmB102  | (TC)24        | F: CGAACCCCTGCTAATGAAC  
R: TGAGCAGAACAGCAGACA  | D4        | 182         | 52        | 2.0             |            |                     |
| BmB106  | (CT)15        | F: GTTTCCCTCAAAAATCTTG   
R: CGGGAGTCGCTGTTGCTTG  | D2        | 221         | 55        | 2.0             |            |                     |
| BmC2    | (CAA)7        | F: CCAAGACTCCCTCAATCACTG  
R: AGCGTCTGGTTTTTCTCTG  | D3        | 242         | 55        | 2.0             |            |                     |
| BmD1    | (TGA)5        | F: CGGAAATCTGTAATGACCTT  
R: TCCCCAGGGAAAGAACAAC  | D4        | 157         | 55        | 2.0             |            |                     |
| BmD4    | (GTC)4(TCA)4  | F: TCTGCTCATCATACAGTC  
R: CAACCAACCAAGACAGCT  | D3        | 284         | 52        | 2.0             |            |                     |
| BmD103  | (CAT)9        | F: AACTGATACAAACAACTATG  
R: TGGGAGGTACTAATCTGCTTG  | D2        | 259         | 52        | 2.0             |            |                     |
| BmD105  | (CAT)6        | F: TTGTGTTACCTTCCGATTTA  
R: TGATGGGGTGATTAAAGAGATG  | D4        | 239         | 52        | 2.0             |            |                     |
Isopropanol PCR product clean up procedure

- Remove diluted primer mix (4 µL + 6 µL dH₂O)
- Add 150 µL 75% isopropanol and mix on a shaker
- Leave on bench for 30 min under aluminum foil
- Spin at 4000 rpm for 30 min and immediately
- Flick off supernatant
- Place plate upside down on clean absorbent paper and pulse spin for 30 sec
- Dry t 70°C for 10 min in PCR machine
- Add Size Loading Solution and 400 size standard and oil before being separated
  by capillary electrophoresis using a Beckman Coulter CEQ 8800 Genetic analysis
  system.
### Table 1 Characteristics for 8 polymorphic microsatellite loci isolated from *Banksia attenuata*

<table>
<thead>
<tr>
<th>Locus</th>
<th>Repeat Motif</th>
<th>Primer Sequences 5'-3'</th>
<th>Dye Label</th>
<th>Anneal temp</th>
<th>Mg conc.</th>
<th>Size range (bp)</th>
<th>No. Alleles</th>
<th>Genbank Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BaA3</td>
<td>(GT)11(GA)9</td>
<td>F: CCACCATACTCATCTTCAG</td>
<td>D2</td>
<td>52</td>
<td>2 mM</td>
<td>136-184</td>
<td>18</td>
<td>EF554582</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: ATCCCAAGGTAAATTCCTTC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BaA112</td>
<td>(GT)2G(GT)10</td>
<td>F: TTCCCCAGAATAGGTCTACTG</td>
<td>D3</td>
<td>52</td>
<td>2 mM</td>
<td>246-264</td>
<td>8</td>
<td>EF554583</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: TTCCACGATTGTGAGATACC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BaC3</td>
<td>(GTT)9</td>
<td>F: TGGCTAGTGGTTTGGTCGTG</td>
<td>D4</td>
<td>54</td>
<td>1.5 mM</td>
<td>183-205</td>
<td>6</td>
<td>EF554588</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: GGTGCTAAAGACCATGATTC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BaC5</td>
<td>(GTT)13</td>
<td>F: TCCGCATTTCAGGATTAGC</td>
<td>D3</td>
<td>54</td>
<td>2 mM</td>
<td>190-216</td>
<td>5</td>
<td>EF554589</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: GGAGCGACCTGGGTACTC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BaB1</td>
<td>(GT)7CC(C)9(AC)7</td>
<td>F: TGGGCACTACTACATCATTTG</td>
<td>D3</td>
<td>53</td>
<td>2 mM</td>
<td>197-243</td>
<td>14</td>
<td>EF554585</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: TTCAAGGGACTCAAGACC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BaB106</td>
<td>(AG)15</td>
<td>F: TGGAAAGTCACTTTTGTATGT</td>
<td>D4</td>
<td>54</td>
<td>2 mM</td>
<td>171-197</td>
<td>11</td>
<td>EF554587</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: GACACTCATGGTTCCTACTAC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BaC8</td>
<td>(CAA)13</td>
<td>F: GCCAGATAGCCTCATCTC</td>
<td>D4</td>
<td>54</td>
<td>2 mM</td>
<td>140-152</td>
<td>5</td>
<td>EF554590</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: TTTGCGATATTCGGTAG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BaC112</td>
<td>(ACA)3CC(ACA)5</td>
<td>F: TGTAAAGTTTTCGATTGAGAG</td>
<td>D3</td>
<td>52</td>
<td>2 mM</td>
<td>127-177</td>
<td>12</td>
<td>EF554591</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: GACCTCGAATGAAGTGGATTG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2 Species present in the post-mine Rocla Jandakot road site, species, family, natural distribution and description (Brown & Brooks, 2002; Hussey et al., 2007)

<table>
<thead>
<tr>
<th>Species used</th>
<th>Family</th>
<th>Native to site</th>
<th>Distribution details</th>
<th>Species used</th>
<th>Family</th>
<th>Native to site</th>
<th>Distribution details</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acacia leephylla</em></td>
<td>Mimosaceae</td>
<td>No</td>
<td>This species is alien to Western Australia</td>
<td><em>Calothamnus quadrifidus</em></td>
<td>Myrtaceae</td>
<td>Possible</td>
<td>Native in its range, and has naturalised elsewhere</td>
</tr>
<tr>
<td><em>Acacia rostelligera</em></td>
<td>Mimosaceae</td>
<td>No</td>
<td>Found on a different sand system</td>
<td><em>Melaleuca raphiphylla</em></td>
<td>Myrtaceae</td>
<td>Possible</td>
<td>Found only in swamps and creek beds.</td>
</tr>
<tr>
<td><em>Acacia saligna</em></td>
<td>Mimosaceae</td>
<td>Possible</td>
<td>Not common in <em>Banksia</em> woodlands, variety of habitats</td>
<td><em>Eucalyptus calophylla</em></td>
<td>Myrtaceae</td>
<td>Yes</td>
<td>Local</td>
</tr>
<tr>
<td><em>Chamelaucium uncinatum</em></td>
<td>Myrtaceae</td>
<td>No</td>
<td>Cultivated variety invades local bush land and can majorly change the plant community it invades.</td>
<td><em>Eucalyptus todtiana</em></td>
<td>Myrtaceae</td>
<td>Yes</td>
<td>Local</td>
</tr>
<tr>
<td><em>Corymbia ficifolia</em></td>
<td>Myrtaceae</td>
<td>No</td>
<td>Found only on the southern coast of Western Australia</td>
<td><em>Regelia ciliata</em></td>
<td>Myrtaceae</td>
<td>Yes</td>
<td>Local</td>
</tr>
<tr>
<td><em>Eucalyptus camaldulensis</em></td>
<td>Myrtaceae</td>
<td>No</td>
<td>Previously used in restoration, now a weed.</td>
<td><em>Adenanthes sp.</em></td>
<td>Proteaceae</td>
<td>Yes</td>
<td>Local</td>
</tr>
<tr>
<td><em>Eucalyptus gomphocephala</em></td>
<td>Myrtaceae</td>
<td>No</td>
<td>Found on coastal plains, naturalized elsewhere</td>
<td><em>Banksia attenuata</em></td>
<td>Proteaceae</td>
<td>Yes</td>
<td>Local</td>
</tr>
<tr>
<td><em>Melaleuca armillaris</em></td>
<td>Myrtaceae</td>
<td>No</td>
<td>This species is alien to Western Australia</td>
<td><em>Banksia menzieii</em></td>
<td>Proteaceae</td>
<td>Yes</td>
<td>Local</td>
</tr>
<tr>
<td><em>Melaleuca lanceolata</em></td>
<td>Myrtaceae</td>
<td>No</td>
<td>Previously used in restoration, has escaped natural range.</td>
<td><em>Hakea sulcata</em></td>
<td>Proteaceae</td>
<td>Possible</td>
<td>Not common in <em>Banksia</em> woodlands</td>
</tr>
<tr>
<td><em>Melaleuca lateriflora</em></td>
<td>Myrtaceae</td>
<td>No</td>
<td>Not local to the Swan Coastal Plain.</td>
<td><em>Kennedia prostrate</em></td>
<td>Fabaceae</td>
<td>Yes</td>
<td>Local</td>
</tr>
<tr>
<td><em>Melaleuca viminalis</em></td>
<td>Myrtaceae</td>
<td>No</td>
<td>Found only in creeks and rocky coastlines.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>