Project Plan SP 2016-015

Is restoration working? An ecological genetic assessment

Plant Science and Herbarium

Project Core Team

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Update requested

Document endorsements and approvals as of June 16, 2020, 9:56 a.m.

Project TeamgrantedProgram LeadergrantedDirectorategrantedBiometriciangrantedHerbarium CuratorgrantedAnimal Ethics Committeenot required



Is restoration working? An ecological genetic assessment

Biodiversity and Conservation Science Program

Plant Science and Herbarium

Departmental Service

Service 7: Research and Conservation Partnerships

Project Staff

Role	Person	Time allocation (FTE)
Supervising Scientist	Dave Coates	0.1
Supervising Scientist	Margaret Byrne	0.1
Research Scientist	Melissa Millar	0.9
Research Scientist	Siegfried Krauss	0.0
Research Scientist	Janet Anthony	0.0

Related Science Projects

Proposed period of the project

June 1, 2016 - July 1, 2019

Relevance and Outcomes

Background

Although a young field, the science of restoration ecology is rapidly growing and being incorporated into natural resource recovery and management strategies at a range of scales worldwide [1]. These restoration activities represent significant investment with a global market estimated at \$100 trillion annually [2]. The recognition of poorly defined success criteria and a lack of long term monitoring have highlighted the need for the development of post implementation empirical evaluations of the quality of ecological restoration activities [3, 4]. Most recently, the field has focused attention on the joint roles that ecological and genetic processes play in ensuring plant populations are self-sustaining, functional and possess the adaptive evolutionary potential that provides resilience to changing environments and persistence in both the short and long term [5-8]. Recognition of how traits affecting reproductive functionality including flowering, fruiting, seed production and seed viability, along with pollinator services, plant mating systems, and patterns of pollen mediated gene dispersal work in concert to shape population demography and levels of genetic diversity is now widespread [9, 10]. This recognition has led to the hypothesis that the most ecologically and genetically viable restored populations will be those where reproductive outputs, plant pollinator interactions, levels of genetic diversity, mating systems and patterns of pollen dispersal most closely mimic those found in natural or undisturbed remnant vegetation. These populations may be more likely to persist in the long term and contribute to effective ecosystem function through integration into the broader landscape. The recent development of theory pertaining to biodiversity on old, climatically buffered, infertile landscapes (OCBILs) adds an extra series of predictions that may yield a more refined understanding of how plant populations function and evolve in OCBILS compared to those on young, often disturbed more fertile landscapes (YODFELs). This body of theory has novel implications for the conduct and outcomes of restoration ecology [11] yet to be tested in large scale restoration programs.

Gondwana Link Ltd are leading an ambitious conservation and restoration initiative that aims to restore native vegetation, providing habitat connectivity and integrated ecosystem function at a regional scale [12]. The project is the largest environmental restoration project ever tackled in Australia and operates across the south west of Western Australia from the wet forests in the west to the dry woodland systems bordering the Nullarbor Plain to the east. There are a number of focus sites within this greater landscape and early restoration activities



have been conducted within the 'Fitz-Stirlings'; a 70 km section of fragmented mallee and woodland remnant vegetation located between the Fitzgerald River National Park and the Stirling Range National Park [13]. Three restoration sites within the Fitz-Stirlings provide ideal experimental locations at which to assess the success of current, state of the art, restoration activities in terms of ecological and genetic viability.

This project aims to improve the adaptive management of already established restoration sites and the planning of future restoration activities by addressing a significant knowledge gap in the field of restoration ecology; that of how well ecological and genetic viability of restored plant populations is secured with different establishment regimes. Additionally, we envisage the Gondwana Link sites allowing us to assess the significance of considering landscape age and fertility (OCBIL theory) in restoration plantings. The project will be conducted at Gondwana Link restoration sites located within the Fitz-Stirling region of Western Australia. However, the outcomes will be of relevance to and will inform broader restoration initiatives in many other landscapes within Australia.

Aims

Specifically, the project aims to assess restoration success through a comparison of ecological (reproductive output, pollinator diversity and behaviour) and genetic (genetic diversity, mating system, pollen dispersal) function within restored populations in relation to that within positive target references of the surrounding undisturbed remnant vegetation.

The project envisages achieving these goals by obtaining measures of genetic diversity and mating system parameters for six target species, present at up to three restoration sites established with differing seed and seedling establishment regimes, for differing lengths of time and with different degrees of consideration of landscape age and fertility. These measures will be compared with populations of the same target species in surrounding undisturbed, remnant native vegetation that will act as positive target reference sites. For two proteaceous species more intensive genotyping of individuals and progeny arrays will allow detailed assessment of pollen dispersal.

Objective 1: Evaluate levels of genetic diversity

For each of the six target species, at each of the restoration sites at which they occur and in three remnant reference sites, 20 individuals will be sampled. This will include the 10 mother plants utilised in Objective 4. DNA will be extracted from all samples and all individuals will be assessed for genetic variation at 12 SSR markers developed for these species.

Objective 2: Evaluate mating system parameters

For each of the six target species, at each of the restoration sites at which they occur and in three remnant reference sites, 10 individual mother plants will be chosen and sampled and seed collected from each. DNA will be extracted from all samples and all mother plants will be genotyped at 6 SSR loci. 20 progeny from each mother plant will be genotyped. Mother and progeny data sets will be analysed.

Objective 3: Evaluate patterns of pollen mediated gene dispersal in Proteaceous nodes

For each of two Proteaceous target species a number of clumps of plants will be selected and all individuals within that clump will be sampled as potential father plants (these will be inclusive of some individuals sampled for Objective 1). Up to 200 progeny per species will be collected from sampled mother plants. DNA will be extracted from all samples and potential fathers and progeny genotyped with 12 SSR loci. The spatial position of all mother and potential father individuals will be recorded via GPS. Paternity of all progeny with known mothers will be analysed.

Expected outcome

This project is significant in addressing a central problem for large scale restoration activities; that of assessing the capability of restoration populations to maintain ecological and genetic processes that mimic those of natural remnant vegetation. This is essential for maximising population demographic and genetic health and for the long term resilience, persistence and integration of restored populations in the broader landscape. However, there is a large degree of uncertainty as to the long term effectiveness of restoration programs [1, 3]. The outcomes of this project will provide practical recommendations on how the ecological and genetic viability of restored populations



may be affected by different establishment regimes. The outcomes will provide a strong basis for cost effective investment of resources in achieving successful restoration through adaptive management of restored sites and for future restoration activities. The project is innovative in employing an integrated evolutionary approach to the field of restoration ecology, contributing to newly emergent theoretical guidelines for the assessment of restoration success as well as in couching research in the broad theoretical context of OCBIL theory which may prove to have important implications for the conduct and outcomes of restoration ecology in the Australian landscape. Research outcomes will identify strategies to develop resilient natural woodland ecosystems that can thrive in environments that are already altered and likely to experience significant ongoing changes.

Knowledge transfer

This project will involve collaboration between The University of Western Australia (UWA), particularly The Centre for Excellence in Natural Resource Management (CENRM), Albany, the Botanic Gardens and Parks Authority (BGPA), Perth, and DPAW. The management team have a strong publication record and publish widely in international peer reviewed journals spanning the fields of ecology, population genetics, restoration ecology, evolutionary biology, molecular ecology and conservation biology. Results of this project will be communicated via publications in peer-reviewed academic journals. Key objectives of the project will be achieved with synthesis of results in a final report that will be distributed to community groups and industry involved in ecological restoration. A public seminar and workshop will cement this communication strategy.

Tasks and Milestones

Ta	Year 1 Year 2 Year 3 Field sampling for genetic diversity and mating system + +
	Field sampling for pollen dispersal
	+
	Microsatellite genotyping
	+
	+ +
	Data analysis
	+
	+
	+
	Identification of factors in restoration success



+

+

Preparation of reports, peer reviewed manuscripts.

+

+

References

- 1. Wortley, L, J Hero & M Howes, Restoration Ecology, 2013. 21(5): p. 537-543.
 - 2. Cunningham, S. 2008: McGraw Hill.
 - 3. Suding, K, Annual Review of Ecology, Evolution and Systematics, 2011. 42: p. 465-487.
 - 4. Miller, J & R Hobbs, Restoration Ecology, 2007. 15: p. 382-390.
 - 5. Monks, L, DJ Coates, T Bell, et al., J. Macschinski and K. Haskins, Editors. 2012, Island press: Washinton.
 - 6. SERI, 2 ed. 2005, Washington, D.C.: Society for Ecological Restoration.
 - 7. McKay, JK, CE Christian, S Harrison, et al., Restoration Ecology, 2005. 13(3): p. 432-440.
 - 8. Broadhurst, L, A Lowe, DJ Coates, et al., Evolutionary Applications, 2008. 1: p. 587-597.
 - 9. Kettenring, K, K Mercer, C Reinhart Adams, et al., Journal of Applied Ecology, 2014. 51: p. 339-348.
 - 10. Hufford, KM & SJ Mazer, Trends in Ecology & Evolution, 2003. 18(3): p. 147-155.
 - 11. Hopper, SD, Plant and Soil, 2009. 322(1-2): p. 49-86.
- 12. Bradby, K, J. Fitzsimons, I. Pulsford, and G. Wescott, Editors. 2013, CSIRO Publishing: Melbourne, Vic. p. 25-35.
 - 13. Jonson, J, Ecological Management and Restoration, 2010. 11(1): p. 16-26.

Study design

Methodology

Objective 1: Evaluate levels of genetic diversity

For each of the six target species, at each of the restoration sites at which they occur and in three remnant reference sites, 20 individuals will be sampled. This will include the 10 mother plants utilised in Objective 4. DNA will be extracted from all samples and all individuals will be assessed for variation at 12 SSR markers developed for these species using the microsatellite enriched next generation sequencing services of the Australian Genome Research Facility (AGRF), Adelaide. Genotyping will be conducted using the services of Murdoch University, Perth. Screening and analysis will involve commonly used software Genemapper (Applied Biosystems), GenALEex [42] and Genepop [43]. Genetic diversity parameters including the number of alleles, the number of private alleles, the mean number of alleles per locus (A), the number of effective alleles (N_e), the proportion of polymorphic loci (P), expected heterozygosity (H_e), observed (H_o) heterozygosity and the fixation index (P) will be assessed for each sampled 'population' within sites using the GenAlEx program.

Objective 2: Evaluate mating system parameters

For each of the six target species, at each of the restoration sites at which they occur and in three remnant reference sites, 10 individual mother plants will be chosen and sampled and seed collected from each. DNA will be extracted from all samples and all mother plants will be genotyped at 6 SSR loci. 20 progeny from each mother plant will be genotyped. Mother and progeny data sets will be analysed using the MLTR [44] software in order to obtain estimates of mating system parameters including, the multilocus outcrossing rate (t_m) , single locus outcrossing rate (t_s) , the apparent level of selfing due to biparental inbreeding $(t_m - t_s)$, the correlation of selfing among maternal plants (r_s) and the multi locus correlated paternity (r_{p_m}) .



Objective 3: Evaluate patterns of pollen mediated gene dispersal in Proteaceous nodes

For each of the two Proteaceous target species, a number of clumps of plants will be selected and all individuals within that clump will be sampled as potential father plants (these will be inclusive of some individuals sampled for Objective 1). Up to 200 progeny per species will be collected from sampled mother plants. DNA will be extracted from all samples and potential fathers and progeny genotyped with 12 SSR loci. The spatial position of all mother and potential father individuals will be recorded via GPS. Paternity of all progeny with known mothers will be analysed using the NEWPATXL [45] and the CERVUS [46] software. Paternity of progeny will be assigned and the spatial distances of pollen dispersal for a given progeny calculated as that between the known mother and the most likely potential father.

Biometrician's Endorsement

granted

Data management

No. specimens

Six.

Herbarium Curator's Endorsement

granted

Animal Ethics Committee's Endorsement

not required

Data management

Raw data sets including microsatellite DNA genotyping data sets will be deposited at publicly available archiving sites such as the Dryad Digital Repository and UWA Research Data Online.

Budget

Consolidated Funds

Source	Year 1	Year 2	Year 3
FTE Scientist			
FTE Technical			
Equipment			
Vehicle			
Travel			
Other			
Total			

External Funds

Source	Year 1	Year 2	Year 3
Salaries, Wages, Overtime	135 489	152 272	103 214



Source	Year 1	Year 2	Year 3
Overheads			
Equipment			
Vehicle	15600	5 200	5 200
Travel	4 000	7 200	
Other	112 730	2000	
Total	265 319	152 272	103 214