

Project Plan SP 2017-022

Do hotter and drier regions harbour adaptive variation for climate change?

Ecosystem Science

Project Core Team

Supervising Scientist	Margaret Byrne
Data Custodian	Margaret Byrne
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Project status as of March 7, 2019, 8:47 a.m.

Approved and active

Document endorsements and approvals as of March 7, 2019, 8:47 a.m.

Project Team	granted
Program Leader	granted
Directorate	granted
Biometrician	granted
Herbarium Curator	not required
Animal Ethics Committee	not required

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Biodiversity and Conservation Science Program

Ecosystem Science

Departmental Service

Service 7: Research and Conservation Partnerships

Project Staff

Role	Person	Time allocation (FTE)
Supervising Scientist	Margaret Byrne	0.0
Research Scientist	Richard Mazanec	0.0
Research Scientist	Katinka Ruthrof	0.0

Related Science Projects

The project is related to SPP 2012-002 Climate resilient revegetation of multi-use landscapes: exploiting genetic variability in widespread species as it is using the same approach. Margaret Byrne is the leader of both projects.

Proposed period of the project

July 1, 2015 – June 30, 2019

Relevance and Outcomes

Background

Rising temperatures and changing rainfall patterns due to climate change can cause large-scale forest dieback and mortality, with major consequences for the maintenance of biodiversity and healthy forest and woodland ecosystems (Allen *et al.* 2005). The Southwest Australia biodiversity hotspot has extensive forest environments that have been subject to drought deaths in recent years. The average rainfall in this region has already declined by 15-20% in the last 30 years resulting in significant crown death of forest (Matusick *et al.* 2013; Brouwers *et al.* 2013), affecting fauna habitat and essential ecosystem services. Rainfall is predicted to decline further (Bates *et al.* 2008) with more frequent and intense droughts (IPPC 2014).

The majority of predictions of how organisms will respond to climate change are based on correlative environmental (niche) models with unrealistic assumptions (e.g. no adaptation, species homogeneity, infinite migration, realised = fundamental niche; Hoffmann & Sgro 2011). While recent efforts have been made to develop more realistic mechanistic models (incorporating dispersal ability - Swab *et al.* 2012; physiological responses - Kearney & Porter 2009; evolution responses to climate - Hoffmann & Sgro 2011), these are restricted to well-characterized model systems, and even in these cases empirical data is limited. To fill this knowledge gap, our project will explore the mechanisms facilitating genetic adaptation and physiological tolerance, that can be utilised to facilitate population persistence in a rapidly changing climate.

The research will focus on *Corymbia calophylla* (*Eucalyptus* sensu lato, family Myrtaceae), an economically and ecologically important forest tree native to the jarrah, tuart, wandoo, and karri forests and woodlands of south-west Australia. This bloodwood, commonly known as marri, is a large tree growing to 40 metres tall in the wild, with very large buds and fruit (Boland *et al.* 2006). Marri is widely distributed from north of Geraldton (28° S) to Cape Riche (34° S), and inland beyond Narrogin (33°S 117° E). It is found on the Swan Coastal Plain and on the Darling Scarp, showing its adaptability to the different environments (traversing major temperature and rainfall gradients). Temperature increases with latitude (c. 24-30°C maximum), while annual rainfall decreases with distance from the coast (c. 400-1200mm) providing a unique opportunity to separate these climatic drivers.

Marri is an important component of the forest ecosystem providing nesting hollows and a food source for endangered cockatoo species. In addition to being an important timber tree, the fruit, seeds, flowers, leaves and wood of marri are all important sources of food for native fauna. The seeds are a major food source for nationally listed threatened birds (Baudin's black cockatoo and Carnaby's black cockatoo) and the maintenance of habitat and food resources is a key factor in forest management practice. In each of the forest and woodland ecosystems where marri is present, it is an important and integral keystone species. Consequently, a decline in its overall health, as we are now experiencing due to drought and disease, will have significant long-term detrimental impacts on ecosystem function. This study has national and international significance for understanding and managing forests for future climates.

Current forest regeneration practice is governed by the Forest Management Plan 2014-2023 that provides for seed sourcing from land management units adjacent to the site based on a broad interpretation of local provenance in widespread semi-continuous species (Byrne et al 2013, Breed et al 2012). Assisted gene migration has also been advocated as a tool for land managers and foresters in managing forests and establishing plantations for future climates (Gallagher et al 2014, Aitken & Whitlock 2013). Unfortunately, there is little information to base guidance for land-managers to implement assisted gene migration. This project will fill this gap and provide a scientific basis for the adoption of assisted gene migration in south-west forests, through a detailed understanding of genetic adaptation and physiological tolerance, to enhance drought-resilience under future hotter and drier climates in marri.

Aims

Understanding genetic adaptation and physiological tolerance are critically important for developing management tools for sustainable and productive forest ecosystems in a drying climate; however, these factors remain relatively unknown. This project will investigate these factors in marri and aims to:

- characterise neutral and adaptive genetic variation to estimate demographic (population size, migration) and evolutionary (adaptation to climate) processes;
- estimate the heritability of plant functional traits associated with growth and resilience, and correlated traits, to determine the propensity for genetic adaptation;
- determine the physiological and molecular capacity to respond to drought to elucidate the mechanisms enabling plants to persist under climate change.

Expected outcome

Assisted gene migration has been suggested as a key climate change adaptation strategy for forests worldwide, and could be readily incorporated into current silvicultural practices in south-west forests through the inclusion of seed from dry adapted provenances in regeneration. This project will provide the scientific evidence required to determine effective assisted gene migration practices in southwest forests for resilience to changing climates, providing a basis for adoption of this practice by decision-makers and acceptance by the community and non-government organisations.

Specific outcomes for the three aims of the project are:

- Identify any population structure and regions with high adaptive variation, providing a foundation for seed sourcing and assisted gene migration to be developed.
- Identification of heritable, functional and ecophysiological traits that are responsive to selection and breeding programs, providing information on the evolutionary direction for future forests.
- Estimation of physiological tolerance of plants to determine capacity to respond to droughts.

Knowledge transfer

Communication is an essential component of this project as significant interest from the research, community, industry and management agencies in forest ecosystem health, gene flow and restoration. We will publish our work in strong peer-reviewed journals such as *Global Change Biology*. We will also present at international and national conferences and workshops such as Ecological Society of Australia and ComBio. Results will also be communicated through local forums that attract significant engagement from scientists, conservation groups, industry, non-government agencies and the general public.

Involvement of Department of Biodiversity, Conservation and Attractions and Forest Products Commission will provide direct technology transfer from this research to forest managers, thus providing a direct conduit to policy development and on-ground management.

Tasks and Milestones

- 1, Recruit Postdoctoral Fellow, and 2 PhD students - December 2015
2. Undertake field work for functional and ecophysiological trait evaluation and genetic analysis - 2016-2017
3. Undertake data analyses for functional and ecophysiological trait evaluation and genetics structure and adaptation - 2017-2018
4. Write papers, complete final report and provide information and recommendations to stakeholders - 2018-2019

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Study design

Methodology

Our conceptual framework utilizes the natural climatic variation and diversity of plant genotypes in south-west Australia (Fig 1) to explore the capacity of plants to respond to climate change through genetic adaptation and physiological tolerance. Our conceptual framework was developed from the findings produced in a pilot ecophysiological research project with CIs Rymer, Tissue and Byrne. In this experiment, seed provenances from the temperature and rainfall extremes of the species were grown under warm (18-32°C) and cool (12-26°C) temperature regimes, and subsequently water was limited to determine the threshold of hydraulic failure. Physiological tolerance (P_{50leaf}) was significantly higher in slower growing warmer climate provenances, than cooler provenances with faster growth rates. Rainfall was not a major determinant of plant growth or physiological tolerance despite the enormous variation among provenances (500-1250mm; Fig. 1). While growing temperature had a significant effect on photosynthetic capacity and plant growth, we found a non-significant response of hydraulics to temperature.

Is this pattern of genetic adaptation and phenotypic plasticity driven by demographic or evolutionary processes? What is the underlying adaptive genetic variation associated with the traits? Is there a heritable component? Is there a continuous cline in the traits along temperature and rainfall gradients? The proposed research will address these questions based on three objectives.

1) Characterise neutral and adaptive genetic variation to estimate demographic (population size, migration) and evolutionary (adaptation to climate) processes

- *Hypothesis 1:* Populations in regions experiencing consistent cool and wet conditions have maintained larger populations harbouring greater neutral genetic variation.
- *Hypothesis 2:* Populations in warm, dry areas are a source of adaptive genetic variation for drought resilience.

Background: It is well recognised that genetic variation is not distributed evenly across the landscape. Geographic and environmental barriers to genetic exchange may subdivide populations. While this may facilitate subpopulations adapting to the local environment (Barton), it could limit the migration of genes from warmer / drier populations to other regions preventing pre-adaption to future climates (Drake et al 2014). At the same time, climatically stable regions may act as a source of genotypes for ephemeral, climatically variable regions (Rymer et al. 2012). Much of our understanding of the importance of genetic variation for adaptive capacity has come from model systems (fruit flies; Hoffmann), crop plants (e.g. maize; Tenaillon et al. 2001), and related species (*Arabidopsis*; Hu et al. 2011). However, for many Australian plants a basic ecological, physiological and molecular characterisation is lacking. The recently published *Eucalyptus* genome (Myburg et al 2014), in combination with advances in next-generation-sequencing technology for population genomics (RAD – Baird et al 2008, ddRAD – Peterson et al 2012), genetic models to elucidate demographic patterns (Hubisz et al. 2009; Hey 2010) and genetic signatures of selection across the genome (Nielsen 2005), has opened the door to ecological and evolutionary investigations. We will establish the patterns of neutral and adaptive variation present within marri to determine the most effective combination of germplasm for use in forest management strategies aimed at ensuring resilience to changing climates.

Methods: Population samples from across the entire geographic and climatic distribution of the species (Fig. 1) have been collected and stored for genetic analyses by PI Byrne DPaW. In addition, seeds are available from FPC for all 170 families represented in the quantitative genetic trials (total 794 samples), providing a large representative sample of the species. We will employ a genotyping-by-sequencing (GBS) approach to genotype thousands of single nucleotide polymorphisms (SNPs) randomly distributed across the genome for large population samples enabling robust estimates of neutral and adaptive genetic variation. GBS reduces the representation of the genome to sequence tags at target cut-sites, which can be highly multiplexed in next-generation-sequencing platforms. Each DNA will be uniquely barcoded to maintain its identity. Sequencing libraries will be produced in-house following the ddRAD methodology (Peterson et al 2012). Quality control will be ensured with the application of robotics (Perkin Elmer Janus Automated Workstation) in the LIEF Robotics Facility (housed at ANU) with blanks and technical replicates on all plates. Libraries will be examined with an Agilent 2100 Bioanalyzer prior to sequencing on the Illumina HiSeq 2000 platform. To obtain high coverage (>20X) of each DNA for approximately 40,000 SNPs we will run the 794 samples on 5 HiSeq lanes. Bioinformatic analyses will be conducted on HIE virtual machine (24 core 128GB RAM), and 'Orange' HPC (Intersect with 3 addition HIE nodes 20 core 256GB RAM each). STACKS and PyRAD will be used to estimate SNPs, with

STRUCTURE and PCoA used to identify genetic relationships. An Outlier analysis using Bayescan v. 2.1 (Foll & Gaggiotti 2008) will be employed to identify the component of genetic differentiation based on diversifying selection. Bayescan is considered to be one of the most robust methods for outlier detection (Narum & Hess 2011; Perez-Figueroa et al. 2010; Savolainen et al. 2013; Vilas et al. 2012), even though the assumption of an island model of allele frequency correlation is not always valid. Neutral loci and loci under divergent selection will be examined separately for associations with geography and climate.

2) Estimate the heritability of plant functional traits associated with growth and resilience

- *Hypothesis 3*: Traits associated with plant structure and resilience will be highly heritable and therefore responsive to selection enabling genetic adaptation.
- *Hypothesis 4*: Growth and photosynthetic traits will have high levels of phenotypic plasticity and therefore responsive to climate change enabling physiological tolerance.

Background: Heritability and genetic variation are the essential ingredients for adaptation to rapidly changing climate. The phenotypic traits expressed by a plant are determined by its genetic make-up as well as the environment. Quantitative genetics partitions the phenotypic variation to estimate the component that is genetically controlled (i.e. heritable). Wood traits have been well characterised for forestry species, showing high levels of heritability and application to breeding programs (REF). Traits associated with leaf and stem resistance to cavitation (embolism) have also been shown to be heritable, with trait variation among related species and populations in different rainfall zones (REF). These structural traits tend not to vary between environments, indicating that genetic adaptation is much more important than phenotypic plasticity. Growth and photosynthetic traits, on the other hand, often show low heritability with trait shifts driven by changes in the climate (temperature & water; Duan et al 2014). Acclimatisation of photosynthesis capacity with warming and elevated atmospheric CO₂ maintains net photosynthesis, thereby allowing plants to allocate resources to other functions and regulate growth.

Methods: Large quantitative genetic trials have recently been established for marri by CI Hardy as part of an existing ARC Linkage (LP120200581) research project with DPaW. Seed was supplied by FPC and additional populations were collected to capture the species distribution. The trials have 18 provenances with approximately 170 families; each had 24 seedlings planted in a randomised blocking design at three sites with contrasting rainfall patterns (Fig. 1). Plantation sites were unused pastures within the natural distribution of the jarrah forest where marri is a dominant canopy tree. This project will measure the rate of growth and a range of plant functional traits at all three sites along the rainfall gradient, targeting provenances that revealed significant genetic and physiological patterns (objectives 1 & 3). Plant height, canopy cover and diameter will be measured each year of the project, and allometric relationships used to estimate plant leaf area, stem volume and above-ground biomass. A subset of trees will be assessed in more detail to explore correlative relationships among functional traits, including leaf and wood properties. Leaf gas exchange campaigns will estimate stomatal conductance, leaf transpiration and net photosynthesis. Leaf and stem hydraulics will estimate the susceptibility to cavitation and therefore sensitivity to drought. The data will be explored using mixed effects models focusing on the response of provenances at the planting sites (highlighting adaptive GxE interactions). Multivariate statistics and cluster analyses will be used to reveal patterns and associations. Heritability of traits, based on the half-sib design, will be estimated by partitioning of the phenotypic variation within and among families at each site and for the combined dataset.

3) Determine the physiological and molecular capacity to respond to climate change

- *Hypothesis 5*: Plants growing under temperature and water regimes found in their native source population will outperform plants from different climates ('local-is-best').
- *Hypothesis 6*: Populations from warm and dry climatic origins will have higher resilience to drought stress.

Background: There is an enormous diversity in how plants respond to their environment. Much of the literature has focused on understanding this diversity of responses with comparisons between plant functional groups (e.g. C3/C4 grasses, Pinto et al 2014), and unrelated species in assemblages (e.g. deserts, Robertson et al. 2009; Patrick et al 2009). More recently there has been growing interest in understanding how these traits have evolved with studies investigating congeneric species and populations of a single species in contrasting climates (e.g. Steane et al. 2014). Plants respond to their environment utilising available resources for growth and reproduction, and maintaining stasis and function during times of stress. A trade-off has been hypothesized where fast growing plants, which invest less in structural components, are more susceptible to stress conditions than slow growing plants (Lewis et al. 2011). Empirical tests have been mixed in support for this hypothesis, which is partly due to variation in the frequency and intensity of stress applied, and partly due to a lack of integration across traits

(Drake et al 2014). An interconnected approach will avoid idiosyncratic results, and will be achieved by sampling from different environments (local and foreign conditions) and stages of stress (base-line well watered through range of water potentials to stress and recovery cycles), with traits sampled across multiple scales from whole plant, leaf level, and cellular to molecular.

Methods: Seed from populations from across the species range are available through PI Devoe (FPC). The 18 provenances represented in the trials will be grown under controlled glasshouse conditions to undertake temperature and water manipulations. Plants will be grown under three temperature regimes (cool 12-26°C, intermediate 15-29°C, warm 18-32°C) maintained to ensure water and nutrients are not limiting. This experiment will enable a test of the 'local-is-best' hypothesis, and identify genotypic (G) and environmental (E) differences, as well as, G x E interactions indicative of local adaptation. A drought cycle will be applied (Duan et al.) to determine both the sensitivity and resilience to drought. The plant responses will be characterised by intensive physiological sampling throughout. Critically this will include sampling at baseline (100% water holding capacity), at regular intervals with water limitation to a defined physiological stress (P50), and following rewatering at regular intervals for a defined period of time. Pots will be weighed and hand watered to maintain similar water availability within treatments. Plant growth will be measured fortnightly as basal diameter and plant height, and at the end of 5-6 months, a final harvest will be conducted to determine leaf, stem and root biomass. Plant leaf traits (size, shape, specific leaf area, stomatal density, and cellular structure) and leaf physiology (photosynthesis, conductance, water potential) will be obtained. In addition, we will explore the response of photosynthetic capacity to temperatures (ACi at 20, 25, 30, 35, 40°C) (Ghannoum et al. 2010), and the leaf susceptibility to water limitation (K_{leaf} at 0 to -60 bars) (Duan et al. 2014).

The molecular response of seedlings from the climatic regions to shifts in growing temperature and drought cycles will be investigated using transcriptome profiling (RNA-seq). Leaf material from all plants and all conditions will be snap frozen with liquid N₂ then stored at -80°C. The molecular processes controlling significant growth and physiological response will be characterized with Illumina RNA-seq. Experimental design will involve 3 growing temperatures (26°C, 29°C, 32°C) X 3 drought periods (baseline, peak, recovery) X 4 climatic regions (warm & dry, warm & wet, cool & dry, cool & wet) X 2 replicate populations (total 72 libraries run on 8 lanes). A reference transcriptome and complete genome sequence for *Eucalyptus* (Myburg et al. 2014) is available for sequence reads to be mapped, and the estimation of gene expression levels and sequence variation. Bioinformatic analyses will be conducted on UWS virtual machines (24core 128G RAM) and the NSW Intersect 'Orange' High-Performance-Compute. This will provide crucial information on the levels of acclimatisation and genetic adaptation to climate change. In brief, the statistical approach will assemble the reads into contigs, which will be mapped allowing large gaps on the annotated *Eucalyptus* genome to generate a reference transcriptome to map the libraries for gene expression. We will employ CLC Genomics Workbench & CLC Server and Trinity software packages.

Biometrician's Endorsement

granted

Data management

No. specimens

Herbarium Curator's Endorsement

not required

Animal Ethics Committee's Endorsement

not required

Data management

Data collected will be made available to the broader scientific community by following emerging journal standards (e.g. Ecological Applications), we will make publicly available data used in all publications on DataOne or Dryad to permit broader use by the scientific community. Sequence data will be deposited in relevant databases

(GenBank, TreeBASE) which are publically accessible. Data from NGS will be deposited with Unite. A data capture system is in place at Hawkesbury Institute for the Environment (entitled 'HIEv') that automates the collection, metadata annotation and backup of sensor-produced data from the HIE field and glasshouse facilities. Such datasets can also be made discoverable to the global community through inclusion in the ANDS 'Research Data Australia' portal. Furthermore, management of project data will be underpinned by responsible practices, in particular through the introduction and use of file naming conventions as well as a strong data policy that is implemented to create an environment of data-sharing amongst researchers within the experiment

Budget

Consolidated Funds

Source	Year 1	Year 2	Year 3
FTE Scientist	0.2	0.2	0.2
FTE Technical	0.1	0.1	0.1
Equipment			
Vehicle			
Travel			
Other	20,000	20,000	20,000
Total			

External Funds

Source	Year 1	Year 2	Year 3
Salaries, Wages, Overtime			
Overheads			
Equipment			
Vehicle			
Travel			
Other			
Total	99,865	96,100	100,540