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Research Matters

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Inside

A golden year for *Nuytsia*, the journal of the Western Australian Herbarium

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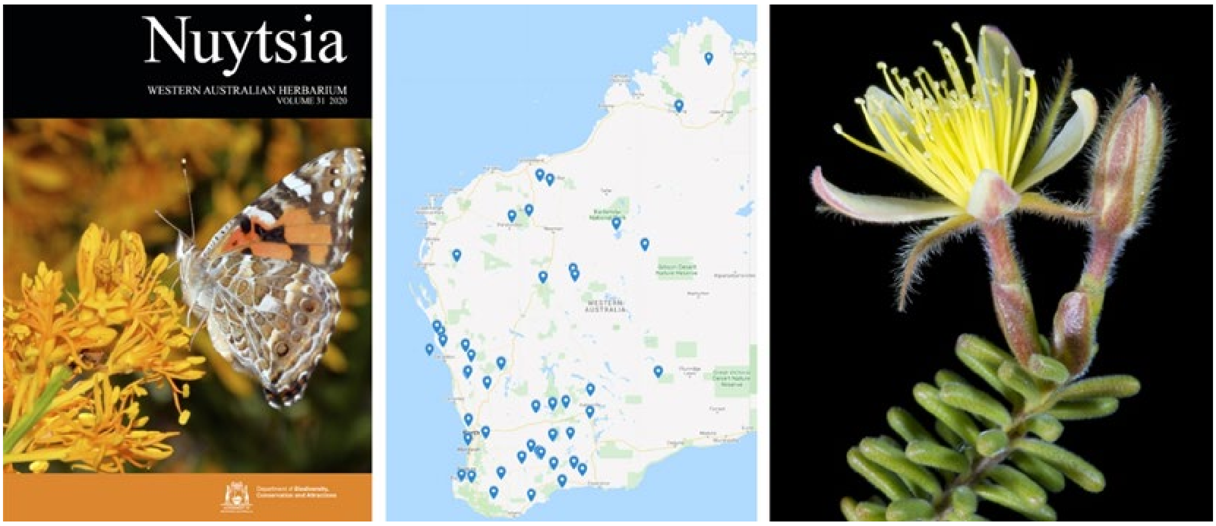
In 1970, the Western Australian Herbarium launched its flagship taxonomic journal *Nuytsia* so that new information on the State's diverse and fascinating flora could be locally published. Named after the spectacular, giant mistletoe *Nuytsia floribunda* (Christmas Tree or *Kaanya* trees), the journal has played a central role in supporting five extraordinary decades of botanical discovery. Over this time, the number of native plants species formally recorded for the State has grown from 5,802 to more than 10,450, of which around one-fifth were scientifically named in *Nuytsia*.

This growth in botanical knowledge has been a collective effort – a vast array of research scientists, curatorial staff, citizen scientists and volunteers have been involved in the discovery of new species, the collection, processing, databasing and curation of herbarium specimens, and the writing, reviewing, editing and publishing of taxonomic manuscripts. To celebrate these achievements and mark the 50th year of *Nuytsia*, 50 new Western Australian species from 50 genera are being published in a golden anniversary edition of the journal.

The 50 species are found in a range of habitats – from the Kimberley to the south coast, including the Perth region and some of the State's most iconic National Parks – and are mostly vascular plants, although two species of algae and one slime mould have been included to highlight ongoing local research on these groups. We are gradually publishing the species each week via *FloraBase* (see https://florabase.dpaw.wa.gov.au/nuytsia/current) and using social media to promote the importance of taxonomy and herbaria and showcase Western Australia's remarkable flora to a wide audience (see https://twitter.com/Science_DBCA and https://www.facebook.com/WesternAustralianHerbarium).

Compiling 50 stories of discovery

To deliver this unique project we drew on our previous experience of setting taxonomic research priorities and expediting the description of new plant species in Western Australia (see Wege *et al*. 2015). We started with a rapid assessment of Western Australia's 'known unknowns', i.e. the list of >1,150 putative new species recognised on FloraBase under informal phrase names. We assessed which taxa were available for study by Herbarium staff and could be progressed to publication within a short time frame, with limited budget, and in parallel with other research commitments. We prioritised rare, threatened or poorly known species to maximise conservation outcomes, drawing on in-house taxonomic expertise or forming new local, national and international collaborations. We also considered species with noteworthy stories of discovery as part of our broader science communication strategy to entertain, educate and inspire our audience.



Left: The cover of the golden anniversary edition of *Nuytsia* brings the flowers of *Nuytsia floribunda* into sharp focus and features an Australian Painted Lady butterfly (*Vanessa kershawii*). Middle: Location of the type (reference) collections for the 50 new species included in the anniversary edition. Right: *Calytrix insperata* (Mundatharrda Calytrix), a recently discovered species from the Kennedy Range National Park. Photo courtesy of Kevin Thiele.

A major challenge was a lack of high-quality Herbarium specimens for many of our short-listed species. Additional material was needed to inform taxonomic decision-making, enable an adequate species description to be prepared, or to serve as type (reference) material. We therefore coordinated a series of field expeditions to obtain specimens, photographs and associated data.

These efforts led to our exciting rediscovery of the stunning rarity *Pimelea crucis* (Tarin Rock Banjine), a species last collected more than 30 years ago and currently known from just eight plants. We also treasured spending time in the bush with local taxonomic legend Barbara Rye to obtain specimens of *Babingtonia peteriana* (Ornate Babingtonia), a geographically restricted species with warty stems that she named for her husband on Valentine’s Day.

Left: Juliet Wege photographs *Kunzea dracopetrensis*. Middle and right: Kelly Shepherd collects *Lechenaultia orchestris* on field trip in which seven of the 50 species included in the golden anniversary edition of *Nuytsia* were collected or photographed.

Our rediscovery of *Pimelea cruciata* (Tarin Rock Banjine) in 2017 enabled its taxonomic distinctness to be confirmed. Just eight plants are known in the wild. Photo courtesy of Juliet Wege.

Top and bottom right: Barbara Rye collects material of *Babingtonia peteriana* (Ornate Babbingtonia) near Three Springs in Western Australia. Bottom left: A beautiful example of Ornate Babingtonia. Photos courtesy of Juliet Wege, Rob Davis and Kelly Shepherd.

While we were able to collect many of our target species, we failed to relocate others, and poor seasonal conditions in some regions of the State forced us to cancel some field trips and change our plan of attack. Despite these challenges, we managed to compile a diverse and interesting mix of new species for the anniversary edition, including showstopping beauties, natural curiosities, recent discoveries, horticultural possibilities, and plenty of rarities.

Taxonomy for conservation

The golden anniversary edition has a strong conservation focus. Of the 50 included species, 42 are conservation-listed in Western Australia, with several known only from a single collection or population, or fewer than 50 individuals. They include *Grevillea hystrix* (Porcupine Grevillea), discovered in 2013 during industry-funded surveys near Koolyanobbing; *Hemigenia diadela* (Mid West Capote), currently known from 17 plants growing near the edge of a small nature reserve near Three Springs; and

Leucopogon kirupensis (Kirup Beard-heath), from a nature reserve near the small town of Kirup in the State's south-west.

These newly named species are all known from single populations. Left: *Grevillea hystrix*; middle: *Leucopogon kirupensis*; right: *Hemigenia diadela*. Photos courtesy of Rob Davis, Mike Hislop and Juliet Wege.

Providing these species with scientific names and descriptions to aid their identification is a critical step that is likely to stimulate surveys and herbarium vouchering and lead to positive conservation outcomes such as the discovery of new populations. This is especially important in view of the escalating threats to our biodiversity in an era of rapid environmental change. Indeed, the pressing need to document our flora has led us to name and describe several species in the anniversary edition from a single herbarium collection. Among them is *Schoenus coultasii* (Annual Bog-rush), a significant discovery made during recent surveys of a mining tenement in a remote part of the Pilbara.

Horticulturally significant discoveries

Several new species in the anniversary issue, while naturally scarce in the wild, show huge horticultural potential. One example is the delicate shrub *Thomasia julietiae* (Juliet's Thomasia), which is confined to a reserve in the Avon Wheatbelt that is surrounded by a sea of farmland. Until recently, this species was known from a single specimen stored in the Natural History Museum in London and we feared it may have gone extinct. It was rediscovered by Mike Hislop, a botanist at the Western Australian Herbarium, who stumbled across it during a bushwalk and had the foresight to collect a specimen. This low, domed plant produces masses of small pink flowers and as such would make a lovely addition to a semi-shaded garden.

Lechenaultia orchestris (Dancing Lechenaultia) is a gorgeous addition to this popular genus. Discovered by wildflower enthusiast William Archer in 2012, it is restricted to a small area of the Mallee bioregion north-east of Ravensthorpe and is one of 13 threatened, rare or poorly known species of *Lechenaultia* recognised in Western Australia. We're thrilled that its horticultural potential is being explored by our colleagues at Kings Park.

Taxonomic research published in the anniversary edition of *Nuytsia* can be used to inform horticulture. Left: *Thomasia julietiae* in its semi-shaded habitat; middle: *Lechenaultia orchestris*. Right: Propagation of Dancing Lechenaultia at Kings Park. Photos courtesy of Kelly Shepherd.

With its attractive silvery green leaves and spectacular clusters of bright yellow flowers, *Geleznowia amabilis* (Kalbarri Yellow Bells) forms an eye-catching sight in the wild. The genus *Geleznowia* is highly sought after by florists as a feature filler and has been commercially harvested from natural bush stands for many years. As *Geleznowia amabilis* is known from only a few scattered populations in the Kalbarri region, it would benefit from being commercially grown so that it is not inadvertently harvested by licenced collectors.

The spectacular, bright flowers of *Geleznowia amabilis*. Photo courtesy of Kelly Shepherd.

The task ahead

Despite 50 years of collective taxonomic effort and downstream conservation benefits, we remain faced with a substantial taxonomic backlog in Western Australia. Furthermore, new species continue to be unearthed on a regular basis through examination of herbarium collections, field surveys and opportunistic collecting, ensuring that our stories of discovery will continue well beyond the golden anniversary of *Nuytsia*.

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*About the authors

Juliet Wege and Kelly Shepherd are based at the Western Australian Herbarium and are both Managing Editors of *Nuytsia*. Both researchers gained their PhD degrees at The University of Western Australia. Juliet's main area of expertise is in taxonomy of triggerplants in the family Stylidiaceae. Kelly's research interests include the 'salt-loving' samphires in the subfamily Salicornioideae (Chenopodiaceae). Kelly is also undertaking a large molecular phylogenetic study of the 'fan-flower' family Goodeniaceae in collaboration with researchers in the United States.

The Science and Art of Regenerating Native Grasslands

Mr Graeme Hand*
Stipa Native Grasses Association Inc.

The impact of unmanaged grazing on native grasslands in the Wannon country south-west of Horsham was recorded by John Robertson in 1853. When he first arrived, Robertson counted 37 different species of perennial native grasses on his run. Sheep were often difficult to find in the long growth. Within 2 years, Robertson observed that bare ground caused by set stocking and the lack of perennial grass recovery gave way to numerous deep erosion gullies across his land, accompanied by the emergence of saline springs.

It follows that if unmanaged grazing was the cause of this degradation then managed grazing of native grasslands may help to reverse it. Management practices that have the following features in common have shown encouraging success in regeneration of grasslands over more than 50,000 ha:

- Promotion of 100% ground cover by growing leaf litter and stabilising it by trampling this litter onto the soil surface
- Management of litter cover so that it can be colonised by beneficial soil organisms to enable it to decompose and provide diverse germination sites
- Regeneration sites in the immediate environment of a viable seed bank

Stipa Native Grasses Association Inc. (STIPA) was formed in 1997 by farmers to promote the profitable management and use of native grasses in agriculture. Many of these farmers have developed or adopted management practices on their properties that regenerate native grasslands. Two examples of non-traditional agricultural practices used for restoring pastures are ‘planned grazing’ and ‘pasture cropping’. Such practices have been confirmed by members of STIPA throughout most mainland states – New South Wales, Queensland, South Australia, Western Australia and Victoria, with farm field trials underway in Tasmania. The practices developed by farmers to regenerate native pastures have been shown to work in many environments and have been corroborated with research. Some of the main practices associated with regeneration of perennial native grasslands using existing seed banks are introduced here.

Seed bank

The most important feature of regenerative management of native grasslands is the soil seed bank. Members of STIPA have been involved in over 400 farm field trials where there has been successful regeneration despite seemingly adverse starting conditions. Thriving pastures and native grasslands have been recruited from very low populations of native perennial grasses ranging from bare ground in cropped areas to less than 5% to 20-30% cover of native grasses. In support of this, soil seed bank research in Victorian grasslands identified a number of grass species that were in the seed bank but not represented in the vegetation (Lunt 1997). Similar situations have been found for alpine summits in south-eastern Australia (Venn and Morgan 2017) and in woodlands in NSW (Lindsay and Cunningham 2009).

Soil surface

Conditions at the soil surface that promote germination and establishment of native grasses include complete litter cover and the capacity for decomposition to take place. These conditions promote water infiltration and storage in the soil profile and can inhibit establishment of weeds. For the best results, the litter from fully recovered perennial grass plants needs to be in contact with the soil surface, ideally, by trampling. Some form of soil disturbance is also required to create suitable germination sites.

Dead plant biomass or litter must be available for decomposition to allow nutrient cycling to become re-established. An important component of a native grassland ecosystem is the fungal content which, in one way or another, allows native grasses to access nutrients. This may be by encouraging decomposer fungi to breakdown litter or as partnerships with fungal symbionts living in and around roots of grass plants. The presence of fungi in soil is often used as an indicator of soil health.

Even where grazing practices have reduced soil biological activity, good management can quickly recover biological activity required for regeneration. Degraded soils often retain components of healthy functioning ecosystems, but they are likely to be in unsuitable ratios. The crucial step is to build the landscape function so that the balance is restored. Along with viable seedbanks, careful management of soil fungal communities is a low cost, low risk technique that can be used to assist regeneration of native grasslands.

Left: Side by side comparison of decomposing grass litter on the left and 'raw' litter on the right. Middle: partially decomposed and well compacted litter cover. Right: fully decomposed leaf litter. Photos courtesy of Graeme Hand.

Trial and error

The first step to learning how to regenerate native grasses from an extant seed bank is to develop small field trials that receive the required management treatments, for example, a high density pulse with animals (1 m^2 per sheep or 3 m^2 per cow), followed by a long period of recovery. Long recovery times are required to allow native grasses to germinate and become established, particularly when soil conditions are not optimal. Native grasses tend to grow and flower at a much slower rate than most exotic grass species so that for most environments in Australia, recovery of about 12 months is necessary. However, as the time required for signs of recovery is dependent on soil, aspect, season and previous management conditions, close observation and monitoring is required.

The key is the ability to be flexible in farm trial areas rather than sticking to a formula of impact and recovery across larger areas of a farm that can possibly have greater environmental or economic impact. The use of 'safe-to-fail' trials or small-scale experimental areas that test an idea are recommended but, to be valuable, they must have a plausible rationale rather than be a 'stab in the dark'.

Weed control

Overuse of perennial grasses, sometimes referred to as overgrazing, creates ideal conditions for many annual weeds to germinate and establish in pastures or grasslands. Good management practices can create healthy yet suppressive soils where highly competitive perennial grass root systems can out compete annuals, such as thistles and annual grasses, and prevent them from becoming established.

Other research has confirmed that regenerative practices can control annuals as well as invasive perennial grasses. For example, seedlings of Serrated Tussock (*Nasella trichotoma*) can be prevented from establishing if the herbage mass of desirable perennial grasses is maintained above 1.5 t dry matter per hectare throughout summer.

Rapid management assessment

Rapid management assessment is quick and simple when using landscape function as the metric. Poor farm management can degrade paddocks and grasslands so that their biological functioning is lower compared to non-farm areas with no active management.

To be considered a successful regenerative grazier, rancher or farmer, the management practices used must result in high landscape function in pastures and grasslands. Regardless of any aspirations of being a regenerative land manager, good grazing practices – regenerative, planned, time-controlled, cell or rotational grazing – requires a simple question to be answered: Is my current management increasing landscape function or not?

The first step for rapid assessment of land function is to look for an 'inverse piosphere'. The piosphere is as an indicator of localised impact of grazing on vegetation and soils. A good example is the ring around a water point which is generally more degraded than further away from the water. Other areas of high animal activity such as mineral salt licks, gateways and sleeping areas, can show the same effect.

When regenerative management is evident a transformation or ‘inverse condition’ has occurred. Here, the basal area of perennial grasses is larger, and litter has higher decomposition near the water point – the opposite of what is normally expected. The first way to complete a rapid management assessment is to inspect the area around the water point of the paddock the animals are moving to next. If management is increasing landscape function the pasture or grassland will be healthier, with more vigorous growth near the water point. The usual causes of a piosphere are that recovery time between grazing events is too short and/or stocking rates are too high. A simple corrective action is to install safe-to-fail practice areas to determine recovery required and then reduce stocking rate and implement a changed grazing plan.

Left: An example of an inverse piosphere with healthy grassland with increased landscape function to the right of the fence and a bare water point with reduced landscape function to the left of the fence. Image from Savory and Butterfield (2006). Right: An example of non-regenerative grazing to the left of the fence compared to roadside vegetation with no management. Photo courtesy of Graeme Hand.

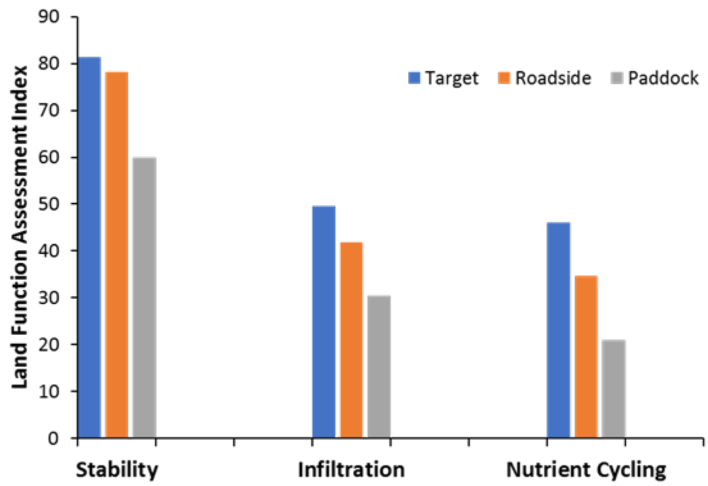
The second way of completing a rapid management assessment is to estimate the landscape function within the farm and on nearby roadside (representing zero management conditions). Both areas can be described as ‘brittle environments’ – land that needs active management to cycle carbon in the low humidity or dry season. When brittle environments are rested for extended periods, their landscape function and biodiversity is reduced. With high rainfall, they form monocultures of perennial grass and have little or no litter decomposition.

As a brittle environment, the landscape function of a paddock must be higher than the roadside for management to be rated as regenerative. Roadside landscape function in brittle environments is usually differentiated by perennial grasses with large basal area and lots of litter in the plants and between the perennial grasses (inter-tussock) with very low or no decomposition.

Upper left: Roadside site with a good litter layer but little evidence of decomposition. Upper right: Sparse perennials with many annuals in the inter-tussock space and representative of little or no decomposition. Bottom left and right: Examples of fungal decomposition of grass litter as a sign of healthy soil functioning. Photos courtesy of Graeme Hand.

Typical paddock landscape function is usually characterised by very few perennial grasses with wide plant spacings more than 1 m, little or no litter present in plants and no litter between the perennial grasses. As with production of a piosphere, the usual cause is that recovery time between grazing events is too short and/or stocking rates are too high. Conversely, in some cases, stock density may be too low to create a good environment for grass seed germination due to paddock design. To increase paddock landscape function, safe-to-fail practice areas are recommended to determine the recovery time and stock densities required for a given area.

Stability of soil relates to how well the soil surface is protected from rain-splash and erosion by wind and water. High levels of water infiltration into soil is important to reduce run-off and to recharge the soil profile with plant-available water. Both of these indicators of landscape function are influenced by soil type, the amount of litter present and if a crust formed by fungi, mosses and lichens is present and intact or not. Nutrient cycling, as a sign of a properly functioning landscape, is characterised by not only the presence of litter but the ability for it to be rapidly decomposed and incorporated into the soil. The amount of perennial vegetation cover present is commonly used an indicator of accumulation of belowground biomass and as a proxy for effective nutrient cycling. Both observed and measurable evidence of differences in landscape function in different types of environments have been demonstrated by members of STIPA.



Comparison of the ideal (target) landscape function for soil stability, infiltration of water and nutrient cycling with roadside vegetation and paddocks as found by members of STIPA.

Regenerating native perennial grasslands in gardens and urban areas

It is possible to regenerate native perennial grasslands in gardens and local patches of degraded grassland in urban areas through mimicking the action of herbivores in grazing systems. To increase germination of perennial grasses the impact of high stock density for relatively short periods of time needs to be recreated followed by an extensive period of recovery, generally, between 9 to 12 months.

This technique has been used to regenerate a lawn of Weeping Grass (*Microlaena stipoides*) in Port Fairy, south-west Victoria, by mowing at low frequency. Similar examples can be found in urban areas in the Sydney basin (see picture below). This practice may be somewhat socially unacceptable as lawns can become very long and small children may become lost! Small safe-to-fail trials away from prying neighbours' eyes are recommended.

In more seasonally humid environments, the use of human foot impact – scuffing and tramping the soil surface – is needed to stimulate germination of native grasses in the soil seed bank. This provides better soil-to-seed contact and breaks up the soil surface. A large group of people will work better than a small group and they must be within each other’s social space to mimic the random behaviour of herbivores at the right stock density. A good excuse to have a garden party. Because regeneration of native grasslands from existing seedbanks is a complex area and there are many unknowns, including the state of the current viable soil seed bank, previous treatments, climate and seasonality, any attempts at regeneration in urban areas will need to incorporate several small safe-to-fail trials to determine what works in your area.

Left: Example of roadside patch of Weeping Grass (*Microlaena stipoides*) responding after occasional council mowing and foot traffic. Top right: Regenerating Box Grass (*Paspalidium constrictum*) growing vigorously after rain. Bottom right: High density pulse with cattle to trample standing litter onto soil surface. Photos courtesy of Tina Bell and Graeme Hand.

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Progress Reports for AFF-Funded Projects

A determination of the horticultural potential of the endangered *Persoonia hirsuta*

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The aims of this project are to:

- Investigate the propagation potential of *Persoonia hirsuta* subsp. 'Yengo NP' for the development of a horticultural cultivar by identifying the factors affecting seed germination, rooting ability of vegetative cuttings, and its initiation into tissue culture
- compare the growth and vigour of juvenile plants from the different propagation methods.

Vegetative cuttings were collected in May 2019 from the nine known extant plants in Yengo National Park, NSW. A total of 350 cuttings were processed from the collection in the nursery at the Australian Botanic Garden Mount Annan. Four hormone treatments were trialled:

- Water soak (control)
- EsiRoot soak
- EsiRoot soak + Clonex green
- EsiRoot soak + Clonex purple

It takes up to 12 months for cuttings to strike. As of January 2020, 26 cuttings have struck with root development across the four treatments (7.5% total strike rate). Currently, strike rate is variable across treatments, ranging from 1-16%. The survival rate of cuttings treated with Clonex purple is very low (18%), indicating a possible toxic effect of indole butyric acid. Cuttings will continue to be monitored and struck plants will be measured for growth over the next twelve months.

In August 2019, 34 stem tips were collected from plants propagated from cuttings and initiated into tissue culture. Plantlets will continue to be sub-sampled to increase replicates for ex-flasking out of tissue culture.

Fruit collection was not possible due to the unfortunate timing of fruit drop and the Gospers Mountain bushfire. This fire burnt through the area where *P. hirsuta* subsp. 'Yengo NP' plants occur. It is possible that this sub-species is extinct in the wild if there is no recruitment and no new unaffected adult plants are found. Consequently, the proposed methodology to assess the germination of this species will be amended to use a smaller fruit collection (approximately 230 seeds) made in 2018 and stored at the NSW Seedbank. Two germination pre-treatments (smoke-water and GA3) will be applied to the seeds and compared with a control treatment.

Persoonia hirsuta subsp. 'Yengo NP' propagated from a vegetative cutting. Photo courtesy of Nathan Emery.

A climatic refugia and the significance of range disjunctions in South Australian eucalypts

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Funding was awarded to explore the evolutionary significance of geographic range disjunctions in *Eucalyptus* species occurring widely in eastern Australia and in disjunct populations in the Flinders-Mount Lofty

Ranges in South Australia. A wide range sampling and molecular (DNA sequence) data was used to determine the evolutionary relationships and divergence times among samples.

Population samples have been collected for three species: *E. albens*, *E. dalrympleana* and *E. macrorhyncha*, including populations from South Australia, Victoria and NSW, covering much of the mainland distribution of each species. Where possible, material from closely related and co-occurring species was also collected to test species boundaries.

DNA sequencing and data analyses

Hybrid-capture and high-throughput DNA sequencing (e.g. Weitemier *et al*. 2014) was used to generate data for this study using a custom bait set designed in-house. We developed two data sets, one comprising chloroplast DNA sequence data including at least 17 genes and associated introns (in many samples, whole chloroplast genomes were recovered); and a separate data set comprising DNA sequences from about 20 nuclear genes. These data are treated separately given the different modes of inheritance (chloroplast is maternally inherited while nuclear DNA is bi-parental), which shapes the evolutionary trajectories of the genes and therefore the inferences that are drawn.

Key findings to date

The chloroplast data, in general, produced well-resolved phylogenies that are incongruent with morphological species boundaries, instead reflecting the geographic origin of the samples. For instance, samples assigned to *E. dalrympleana*, *E. viminalis* and *E. bicostata* collected in South Australia fall together in a well-supported clade, while samples of the above, collected from eastern Australia form a clade that is sister to the 'South Australian' lineage. Molecular dating analyses suggest the divergence of these two groups may be in the vicinity of 2 million years, which likely predates the age of the included species (Thornhill *et al*. 2019). The strong geographic pattern in these data may be explained by localised inter-specific hybridisation and introgression, leading to chloroplast capture (i.e. transfer of one species chloroplast into another species), a process widely reported among *Eucalyptus* (e.g. Flores-Rentieria *et al*. 2017; Alwadani *et al*. 2019). The relatively deep divergence between the South Australia-eastern Australia lineages could be explained if the chloroplast type in a given region has been inherited over millions of years among species (including presumably extinct ones) that are capable of hybridising.

In each of our study species, the nuclear data supports the distinctiveness of the South Australian samples. Our analyses suggest that the South Australian and eastern Australian lineages separated approximately 800-1400 generations before present. While noting there are difficulties in estimating generation time (i.e. the number of years from seed to seed), applying reasonable values (50-100 years/generation) yields divergence time estimates pre-dating the last glacial maximum (approximately 20,000 years before present) and potentially pre-dating the onset of the last glacial period (100-120,000 years before present) (Harle *et al*. 2002). These estimates support the *in situ* survival of our study species in the Flinders-Mount Lofty Ranges through periods of highly unfavourable regional climate consistent with a refugial status for the region.

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Is mitochondrial function the key to improving cryopreservation of threatened Australian flora?

Bryn Funnekotter
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Background

This project aims to advance the fundamental science of metabolic function that impacts the successful cryopreservation of threatened Australian plant species.

Cryopreservation is the safest and most effective long-term conservation method of storing valuable species and genotypes, involving storage of living biological material in liquid nitrogen (LN). The integrity of mitochondrial function within cells is essential for the successful recovery of cryopreserved material but there has been very limited investigation in plants. The energy stored in ATP produced by the mitochondria is vital in almost all aspects of cell metabolism, and it is of particular importance for cryopreservation due to its role in providing energy for repairing damaged DNA, the production of new proteins and lipids, and the energy to resume normal cell division and growth after storage.

The project will increase our understanding of the stresses experienced by Australian plants during cryopreservation. Specifically, the characterisation of mitochondrial function and integrity in plant tissues will be pioneered as a novel approach to the development of species-specific cryopreservation protocols for some of Australia's endangered and critically threatened rainforest species. This project will use new non-invasive techniques to assess mitochondrial function during the cryopreservation process in threatened Australian species. This includes the ASTEC Global Technology Q2 Oxygen Sensing Technology to assess metabolic function after cryopreservation; confocal microscopy with mitochondria-specific fluorescent probes to visualise mitochondrial damage within cells during cryopreservation; and the Seahorse XFe96 Flux analysis, to gain specific insight into the toxic nature of CPAs effect on the various components of mitochondrial function.

Progress to date

The initial study utilising the Q2 yielded some interesting and valuable insights, confirming our hypothesis that the cryopreservation process severely affects mitochondrial function. While there were some difficulties surrounding bacterial contamination in the early trials on the Q2 slowed progress, a robust method has now been developed for analysing shoot tips on this new instrument. The initial study was completed on two Australian species, *Androcalva perlaria* and *Anigozanthos viridis*, both showed significant decreases in metabolic activity after cryopreservation.

Another point of interest this study highlighted was that the shoot tips that visually looked dead still showed considerable metabolic activity during the first couple of days post-cryopreservation, an interesting finding that needs further investigation to see if these shoot tips can be saved. Work is continuing utilising the Q2 to develop cryopreservation protocols that reduce metabolic stress to the plants, and to assess a wider range of species to see the extent of damage that can occur to mitochondrial function during cryopreservation.

Work has only just begun on the Flux analysis trials, limited by the process of starting viable cell cultures, particularly for native species with no established protocols. Currently carrot cell cultures, carrot somatic embryos and *Arabidopsis* shoot tips have been used to test the Seahorse

Flux instrument, establishing some base line information and procedures for further testing. Optimisation of the plant tissue material, amount of plant material needed, growth medium, pH and temperature is still essential before further testing can begin on the effect CPAs have on mitochondrial function, aiming to provide some valuable insights into the findings from the Q2 trials.

Development of molecular markers for resistance to myrtle rust in Australian Myrtaceae

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Background

Most Australian myrtaceous species have been shown to be vulnerable to infection by the fungal pathogen *Austropuccinia psidii*, causal agent of myrtle rust. Although a species may be susceptible to infection by *A. psidii*, a small proportion of resistant individuals are usually found in genetically diverse wild populations when tested in controlled inoculations. These variable responses suggest that resistant individuals, when identified, may be useful for replanting and breeding programs and to develop molecular markers for germplasm screening.

Previous RNA-seq analysis has identified candidate genes that are associated with resistance within *Syzygium luehmannii*. These candidate genes, both recognition receptors, need further computational resolution due to the well-known problem in assembling immune receptor genes. Newly developed software, specifically for aligning short reads from immune receptor genes will be used to refine the specific gene assembly. With this data, gene specific primers can be designed for testing resistant versus susceptible genotypes of *S. luehmannii*.

The aim of the project is to develop primers for use across a range of Myrtaceae that will characterize plants as either resistant or susceptible to myrtle rust.

Computational characterisation

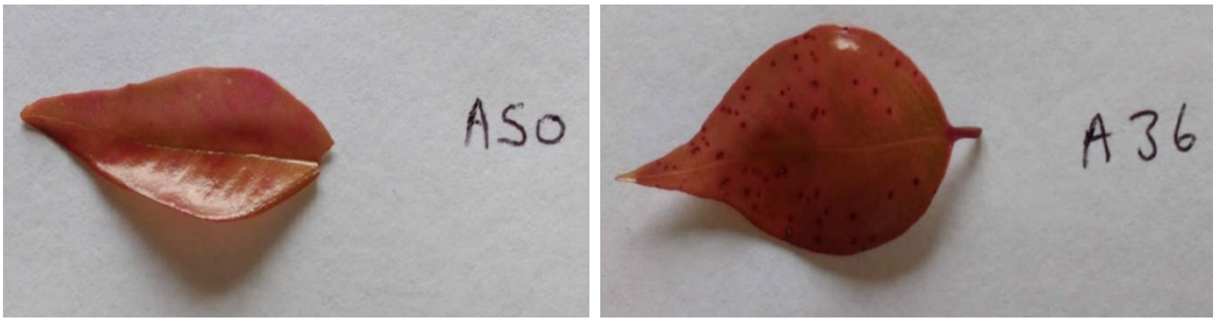
Existing RNA-seq data from inoculated plants was used to make targeted new assemblies of the currently identified genes for resistance within *S. luehmannii*. This step was completed using custom python scripts run on the University of Sydney high performance computer. Outputs were multi-sequence fasta files for both putative resistance gene (APR for *A. psidii* resistance) and receptor-like kinases (RLK) previously noted for differential expression in resistant plants. Based on the longest assembled transcripts in resistant plants, primers were developed and tested.

Primer development and testing

With a comprehensive assembly of the receptor genes, specific primers were developed and tested first on a single resistant and susceptible plant cDNA. RNA-seq differential expression indicated that the RLK was absent in susceptible plants pre-inoculation and that the APR gene was upregulated in resistant plants at 24- and 48-hours post-inoculation.

Screening for phenotype

Plants of *S. luehmannii* (n = 50) were inoculated under controlled conditions and scored for response after 10 days. A single plant with hypersensitive response (HR) will be sequenced with Oxford nanopore Minion® and long read assembly mode. This along with the further primer testing will permit a more accurate diagnostic assay being developed.



Response to controlled inoculation. Left: highly susceptible (A50) and right: hypersensitive response (A36) at 10 days post inoculation. Photos courtesy of Peri Tobias.

What Research Were We Funding 25 Years Ago?

See http://aff.org.au/results/grant-summaries/ for further details of these and other research projects funded by the AFF.

Cloning and selection of banksias

Prof. Margaret Sedgley
Department of Horticulture, Viticulture and Oenology, University of Adelaide, South Australia; funded in 1993 for $3,000 and in 1995 for $3,000

Today it is possible to find *Banksia* as a cut flower at almost any time of the year and in a variety of colours – red, yellow, orange, green and cream. There are about 20 species that are traded as cut flowers with species most suited to cutflower production being *B. hookeriana*, *B. coccinea*, *B. baxteri*, *B. prionotes*, *B. menziesii*, *B. speciosa*, *B. burdettii*, *B. attenuata* and *B. grandis*. Banksias grown for the cutflower trade are cultivated in Western Australia, South Australia, Victoria, Queensland and

New South Wales. From a survey done in 1999, there were an estimated 1.9 million Banksia stems produced each year (Department of Agriculture and Food 2007). The successful cultivation of many of these species can be traced back to the pioneering work by Prof. Sedgley funded, in part, by the AFF.

The research involved cutting propagation and micropropagation of four species of *Banksia*. For cutting propagation, newly grown terminal and sub-terminal shoots, approximately 10 cm long, were selected after they had started to harden. Half of the leaves were removed from the cutting and the remaining leaves were cut in half. The base of the cutting was recut under water and wounded by two longitudinal cuts of 1.5 cm length. The base of each cutting was held in varying concentrations of indole butyric acid (IBA) for 5 seconds before transferring to small pots with equal volumes of peat, coarse sand and perlite. Prepared cuttings were incubated with bottom heat and misting for 5 seconds every 10 minutes.

Buds on explants of *B*. *ericifolia* and *B*. *menziesii* failed to develop into shoots. For *B*. *coccinea*, new shoots and roots developed although attempts to transfer plantlets were unsuccessful.

Using newly emerging micropropagation techniques, *B*. *coccinea* and *B*. *spinulosa* var. *collina* were successfully multiplied *in vitro*. Nodal segments from 6-12 month old seedlings were grown using modified half strength Murashige and Skoog medium supplemented with benzyladenine and IBA. Shoots of *B*. *coccinea* were rooted *in vitro* using IBA or naphthalene acetic acid, but the plantlets could not be weaned successfully from the culture flask to the glasshouse.

Based on this research, three cultivars for cut flower production were registered with the Plant Breeders Rights Office in Canberra – Waite Orange, Waite Crimson and Waite Flame. Waite Orange is a hybrid of *B*. *hookeriana* and *B*. *prionotes* and has peak flowering in May. Waite Crimson is a late spring-flowering selection of *B*. *coccinea* and Waite Flame is an early spring-flowering selection of the same species.

References

Department of Agriculture and Food (2007), The *Banksia* production manual. Department of Agriculture and Food, Western Australia, Perth. Bulletin 4710.

Sedgley M (1998) *Banksia*: new proteaceous cut flower crop. *Horticultural Reviews* 22, 1-25.

Tynan KM, Scott ES, Sedgley M (2000) *Banksia* propagation. *In-vitro* multiplication of *Banksia* species. *Australian Plants* 21, 79-82.

Top left: Ten stem bunch of *Banksia hookeriana* (Hooker's Banksia) (image from: azaleaflowers.com.au/products/banksia?variant=31825981210659); top right: eight stem bunch of *B. menziesii* (Firewood Banksia) (image from: premiumgreensaustralia.com/portfolio/banksia/); bottom left: *B. coccinea* (Scarlet Banksia) (image from www.agric.wa.gov.au/nursery-cutflowers/banksias-cutflower-production); bottom left: *B. baxteri* (Baxter's Banksia) (image from en.wikipedia.org/wiki/Banksia_baxteri).

Development of Native Bluebells for broadscale landscaping

Iain Dawson and Brian Sindel
Division of Plant Industry, CSIRO, Canberra, ACT; funded in 1993 for $3,000

The genus *Wahlenbergia* in the family Campanulaceae has about 260 species worldwide. This is a widespread genus but is found mostly in the southern hemisphere, particularly in Africa. Plants are annual or perennial herbs and occasionally woody shrubs, with simple leaves and blue to purple bell-shaped flowers, usually with five petals. There are 26 species in Australia with the greatest concentration of taxa in the south east of the country (Smith 1992).

One of the most widespread and well-recognised species in Australia is *W. stricta* (Tall Bluebell). This species can be found throughout south eastern Australia extending from the Carnarvon Range, Queensland, south to southern Tasmania and west to Eyre Peninsula, South Australia. It can be found over a wide altitudinal range from sea level to about 1,500 m. It is common in forest, woodland, scrub and grassland, typically growing amongst other herbs. *Wahlenbergia communis* (Tufted Bluebell) is also widespread throughout south eastern Australia but is absent from Tasmania. This species also occurs in a variety of vegetation types and is often found in disturbed sites, particularly along roadsides.

In the study that was funded by the AFF, plant specimens and seed of two species of Native Bluebells (*Wahlenbergia stricta* and *W. communis*) were collected from roadside populations from northern NSW, the Western Plains and central and southern Victoria in December 1992. Data recorded included habitat, population size, plant height and habit, and aspects of the flowers and flowering. *Wahlenbergia stricta* was generally found in woody or rocky sites on roadside embankments while *W. communis* occurred mainly on the gravel road verges immediately adjacent to bitumen surfaces.

Based on the variation in morphology, flowering characteristics and seed production, *W. stricta* and *W. communis* were selected for their potential for use in broadscale landscaping. Plans were made for existing accessions to be tested for germination requirements and were to be grown under controlled environment conditions for comparative evaluation of their physiology.

Although this research was never fully reported for the AFF, the suitability of these and other species of Australian *Wahlenbergia* for revegetation is evident (for example, see Land for Wildlife; Greening Australia). Many species are drought- and frost-tolerant and can grow in a variety of soil types. Commercial cultivars selected for use in a wide range of landscaping situations include *Wahlenbergia stricta* 'Blue Mist' which has a double royal blue flower. Other commercial cultivars include flowers that are pale blue (*Wahlenbergia stricta* 'Sky Mist', white (*Wahlenbergia stricta* 'White Mist') and pink (*Wahlenbergia stricta* 'Fairy Mist'). Seed of *Wahlenbergia* are not commonly available but, when they can be purchased, germinate within 3-4 weeks. Propagation from cuttings is also possible. Plants and plantlets are fast-growing, make a very good ground cover in sun and semi-shade and can resprout after fire once the plants are mature.

Left: *Wahlenbergia communis* (Tufted Bluebell) (image from: keys.lucidcentral.org/keys/v3/scotia/key/Plants%20and%20Fungi%20of%20south%20western%20NSW/Media/Html/Wahlenbergia_communis.htm); right: *W. stricta* (Tall Bluebell) (image from: en.wikipedia.org/wiki/Wahlenbergia_stricta#/media/File:Wahlenbergia_Stricta_Red_Hill.jpg).

References

Smith PJ (1992) A revision of the genus *Wahlenbergia* (Campanulaceae) in Australia. *Telopea* 5, 91-175.

Land for Wildlife (wildlife.lowecol.com.au/wp-content/uploads/sites/25/Propagation-and-Revegetation.pdf)

Greening Australia (www.greeningaustralia.org.au/wp-content/uploads/2017/11/FACT-SHEET_Wahlenbergia_gracilis.pdf).

About the Australian Flora Foundation

The Australian Flora Foundation is an Australian not-for-profit charity dedicated to fostering scientific research into Australia's flora. It is totally independent. All members of the Council and the Scientific Committee give their time freely as volunteers.

Each year the Australian Flora Foundation provides funding for a number of grants for research into the biology and cultivation of the Australian flora. While the grants are not usually large, they are often vital in enabling such projects to be undertaken. Many of the researchers are honours or postgraduate students, and their success with an Australian Flora Foundation grant hopefully stimulates their interest in researching Australia's unique and diverse plants throughout their careers. This work is only made possible by the generous support of donors and benefactors.

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