



*A charity fostering scientific research into
the biology and cultivation
of the Australian flora*

Research Matters

Newsletter of the Australian Flora Foundation

No. 34, July 2021

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Australian Flora Foundation 40th Anniversary

"Please accept this invitation to attend the Inaugural Meeting of the Australian Flora Foundation at The University of Sydney, on Friday, 14 August 1981 at 2.30pm.

At 2.30 pm a Reception will commence and refreshments will be served. A short informal meeting will follow and the Foundation will be formally proclaimed by Dr Lloyd Evans, President of the Australian Academy of Sciences. The Hon. Neville Wran QC, Premier of New South Wales, and a number of selected speakers will then briefly address the Meeting."

The text above headed an invitation to attend the inaugural meeting of the Australian Flora Foundation. The records I have of the early period state that the concept of a Foundation arose from a Symposium held at (then) Hawkesbury Agricultural College in 1978. There were meetings of a Steering Committee/Provisional Council in 1980 and early 1981 to scope out the workings of the Foundation. This preparatory work culminated in the August 1981 launch, timed to coincide with the International Botanical Congress held in Sydney in August 1981. The people involved in getting the Foundation up and running were a mix of academic and government scientists, horticulturists from both academia and from industry, and members of the Society for Growing Australian Plants, who gave and still give (under various names such as the Australian Plants Society) strong support to the Foundation.

The objective of the Foundation was stated as *"to foster research into the biology and cultivation of the Australian flora"*. It was to be a national body, with invited membership (annual fee, \$1), a governing Council, and a scientific Committee to review research applications, which would allow tax deductibility for donations.

Several milestones followed in short order. Tax deductibility status was achieved by April 1982, with the stipulation that a Scientific Committee (which had been formed and vetted by CSIRO) approve research expenditure. Formal incorporation was achieved as a Company in 1983, and then as an Incorporated Association in 1988. By 1984, invited membership was dropped and membership was opened to the public (143 members by April 1984). By 1986, the Council decided that the financial position of the Foundation was sufficiently strong to allow a call for research applications to be issued for grants commencing in 1987. The call for grant applications has been issued annually ever since. Communication with members was by Annual Reports early on; in 1983, a Newsletter was established as an alternative way of doing this, and it is now issued twice a year. In 2004, the Foundation established a website, courtesy of Peter Goodwin and Val Williams.

From its inception, the purpose of the Foundation has always been to raise money for research and distribute it as grants. Peter Goodwin, in his Presidential Report of 2012, conveniently divided the grant activity into three phases. In the first (pre-bequest) phase, incoming funds came from donations by members and the public, and membership fees. Three grants were supported in the initial 1987 round, and by 1992, the Foundation had distributed \$30,000 in grants, and was

averaging two grants, totalling \$4,000, per year. A second (small bequest) phase opened from 1993 onwards, with the Foundation receiving some smaller bequests (Bowden, Armitage) and one larger bequest (Carver), and money from external sources via the efforts of Malcolm Reed (RIRDC; \$34,450; Lord Mayor's Bushfire Appeal; \$56,336). Numbers of grants, and grant size, increased as a result. A third phase began in 2000, when, with assistance from Ross Smyth-Kirk, money from bequests was consolidated into three Managed Funds. The aim was to allow capital growth of the financial assets (then \$591,500), whilst using the dividends to support research. By 2012, the Foundation had distributed over \$500,000 in grants, and was awarding three or four grants, totalling \$40,000, per year. Capital growth resulted in assets in the Research Fund reaching \$953,000 by 2020. Overall, to 2021, the total amount spent on grants reached \$986,000 spread over a grand total of 137 grants. The average number of annual grants was now four, totalling \$49,000, per year.

The Foundation is about to embark on a fourth phase of its growth, based on the very generous bequest from former President, Malcolm Reed. The details of how this will run are still being worked out. In summary, the assets of the Research Fund will be in excess of \$4,000,000, and the annual granting budget will probably exceed \$200,000.

In relating the story above, I am struck by the courage and vision of the founders seeing the need for a fund-raising and grant-distributing body to focus on native flora and seeing through the actual formation of the Foundation. The story of its subsequent growth (patchy at times) over 40 years, and the extent of support to scientific research in the native flora that has been made possible, fully justifies their vision. The Foundation has contributed just short of a million dollars to scientific research into Australian native flora since its launch. If the Foundation has not been formed, while some of that research would have been funded by other means, a fair proportion of it would not have been funded.

Then in addition to being thankful for the vision of the founders, we should also be grateful to the donors (the various Australian native plants societies have consistently been our major donors), the members whose annual fees have largely covered administration costs, the scientists who have served on the Scientific Committee, and the scientists who have applied for funds and carried out the research. Then there are the volunteers who have served on the Council of the Foundation; their time is given freely and has meant that administrative costs have been minimised.

I have not given an account of the impact of the research funded by the Foundation in the account above but hope to do so in a later issue of the Newsletter. I think all members, Councillors, researchers, and donors can be proud of what the Australian Flora Foundation has achieved to date. And we can look forward to a brighter (and busier...) future.

E. Charles Morris
President and Treasurer
Australian Flora Foundation
26 July 2021

Can nursery growing medium influence translocated plant growth and flowering?

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Introduction

Global pressures from urbanisation and a changing climate are increasing the need for a suite of *in-situ* and *ex-situ* conservation tools to protect biodiversity. *In-situ* refers to conservation of plants where they occur naturally and *ex-situ* refers to plants being stored in protected collections, such as nurseries, seed banks or managed collections.

One technique which straddles the divide between *in-situ* and *ex-situ* conservation, and is increasing in use, is translocation. It is defined as the movement or direct transport of plants or plant material from one area to another to benefit a species or ecosystem (IUCN 2013).

According to Commander *et al.* (2018), the goals for plant translocation are most commonly for:

- Introduction – where plants are introduced into areas where they previously did not exist
- Reintroduction – where plants are re-introduced in places where they used to occur
- Augmentation – where existing populations are bolstered with the addition of new plants

Translocation methods can involve whole plants (where plants are literally dug up and relocated) or direct seeding (where seeds are scattered or introduced into the new environment). However, translocation most commonly relies on propagation of plants in nurseries before they are planted as tube stock.

Despite increasing popularity (Silcock *et al.* 2019), translocation is resource-demanding and success rates are uncertain. One method for potentially increasing the rate of success is by manipulating the growing conditions of plants while in the nursery. This method has been thoroughly investigated for application in commercial forestry and silviculture but less so for translocation of threatened plants.

Case study using *Hibbertia spanantha* – testing nutrient loading and provenance soil inoculation

The critically endangered *Hibbertia spanantha* was used as a case study species (Doyle *et al.* 2021). We manipulated two nursery propagation techniques to determine if the changes affected plant growth and flowering. The two methods used were varied rates of fertiliser addition ('High' and 'Low') and inoculation of standard nursery potting mix with 'home soil' (addition of 50% soil from the site where plants are intended to be introduced). These two methods are often referred to as nutrient loading and provenance soil inoculation, respectively.



Above: Critically endangered *Hibbertia spanantha* (Julian's Hibbertia). Image provided by the author.

Why nutrient loading?

Nursery-based nutrient loading can facilitate growth and nutrient storage in woody perennial seedlings (mostly shown for northern hemisphere *Populus*, *Quercus* and *Pinus* species). This method can improve the competitive ability of transplants and increase stress resistance with the consequence of reducing requirements for post-planting care (Timmer 1997; Schott *et al.* 2016). In Australia, research investigating nursery-based nutrient loading to confer benefits to transplants is uncommon but field application of fertiliser for restoration and silvicultural purposes does occur, often resulting in varied responses driven by environmental conditions (Stoneman *et al.* 1995; Rokich and Dixon 2007). Consequently, a greater understanding of the positive or negative impacts of nutrient application pre-planting is needed as clear guidelines are limited.

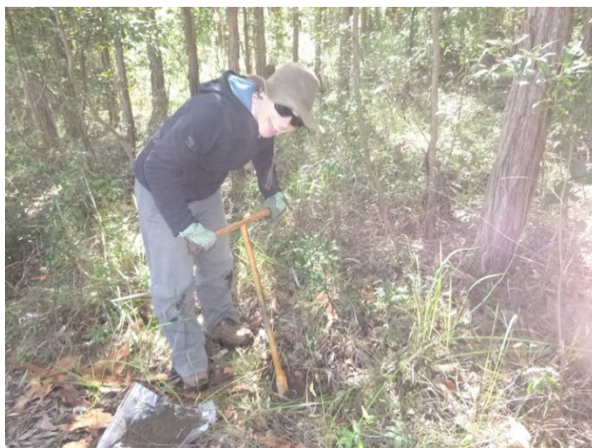
Why provenance soil inoculation?

Promoting mutualistic relationships with soil microbiota, such as mycorrhiza and rhizobacteria, at the pre-planting stage has been found to increase establishment success and reduce transplant shock in threatened plant translocation (Haskin and Pence 2021). Mutualistic relationships afford plants access to limited nutrients, particularly phosphorous and nitrogen, and micronutrients such as zinc and copper. They also confer greater absorption of water to afford drought and pathogen resistance. For management of threatened flora, inoculation is sometimes critical for propagation of species with symbiotic associations such as orchids (Reiter *et al.* 2018). Many plant species have mycorrhiza-specific relationships based on geography or climate (Gemma *et al.* 2002; Bothe *et al.* 2010).

Provenance soil inoculation is a cost effective and simple method of introducing species-specific or location-specific microbiota and may provide similar results to the application of cultured rhizobacterium (Michaelis and Diekmann 2018). This method may also introduce unidentified co-associations such as helper bacteria (Duponnois and Plenchette 2003), increase plant tolerance to biotic stress and immune response to pathogens (Chialva *et al.* 2018), expedite acclimatisation to the home environment before planting, and reduce the risk of microbial competition post-planting (Haskins and Pence 2012). Conversely, home soil inoculation can also potentially introduce soil borne pathogens (Mendes *et al.* 2013).

Does nutrient loading and provenance soil inoculation change plant growth and flowering?

Our study, although small, found that during propagation, plants supplied with low levels of nutrients or raised with provenance (home) soil grew better compared to those receiving high levels of nutrients (Doyle *et al.* 2021). However, these differences did not persist in the field. At 11 months post-planting, plants treated with provenance soil had growth rates that were no different to the other two treatments. We did find that plants that received high levels of nutrients or were grown in provenance soil produced more flowers indicating that provenance soil could confer a flowering advantage akin to addition of fertiliser.



Left: The author planting of critically endangered *Hibbertia spanantha* (Julian's Hibbertia) in remnant Turpentine Ironbark Forest, Sydney. Image courtesy of Belinda Pellow. Right: The author counting flowers of translocated plants. Image courtesy of Simon Dunne; both images provided by the author.

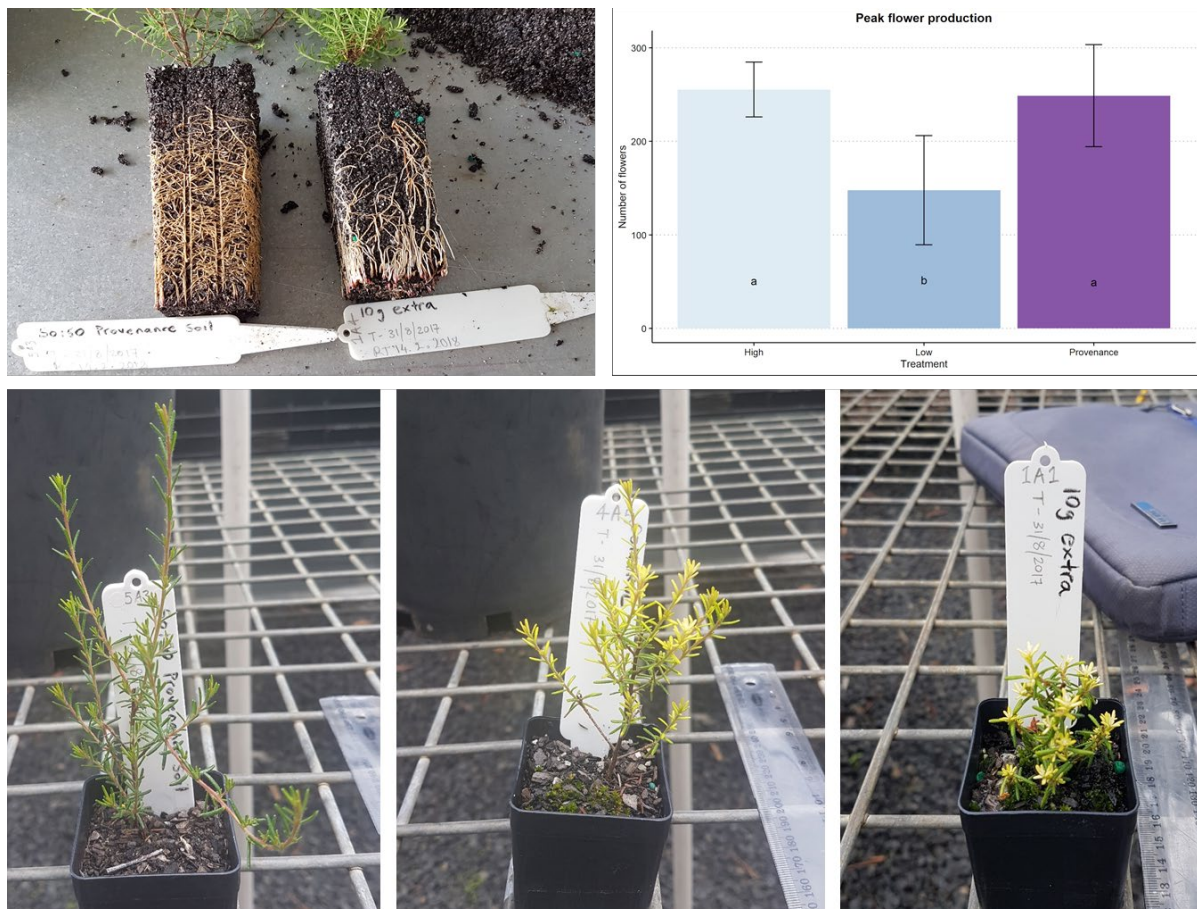
Anecdotaly, the structure of the root systems of plants grown with high nutrient levels were different compared to plants receiving low nutrients or grown in provenance soil. Nursery plants with access to high nutrients developed chlorotic growth although the effects did not persist past 6 months of age.

Should we consider propagation when growing plants for re-introduction into the wild?

Based on resource demands required for translocation when used as a conservation tool, factors which may increase the likelihood of plant survival and reproduction should be incorporated at all stages, including propagation. Our

study demonstrated that plants adapted to nutrient poor conditions can be grown successfully using potting mix supplemented with home soil and under lower nutrient regimes than conventionally used. In addition to positive outcomes for the plants themselves, reducing the costs associated with propagating media and fertilisers would be beneficial for conservation projects which are often community-driven and poorly or intermittently funded.

Our study revealed that nursery propagation culture can and should be incorporated into the design and planning of translocation projects and that simple and cost-effective nursery practices are well placed to be part of the toolkit, providing ethical and phytosanitary measures are addressed.



Top left: Differences in root structure between 6-month-old plants of *Hibbertia spanantha* grown in 50% provenance soil and 50% standard potting mix (left) and plants supplied with high nutrients (right). Top right: Peak flower production after translocation of nursery plants into the field. The bars indicate the average number of flowers produced for each treatment with the letters 'a' and 'b' showing which treatments were statistically similar (High and Provenance (home soi)) or different (Low). Bottom: Chlorotic growth in plants grown using different media or nutrient treatments; Provenance (left), Low (middle) and High (right). All images provided by the author.

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***About the author**

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Indigenous plants bring culture, beauty, and beneficial insects into our parks and gardens

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“[Indigenous plants] tell stories about the cultural belonging of Indigenous peoples and allow a portal into the rich cultural and ecological knowledges held by Indigenous peoples.”

Zena Cumpston in 'Indigenous Plant Use' (Cumpston 2020)

Look closely at nature

I have a rather unusual request. If you can and have a minute to spare, would you have a detailed look at the four images in the figure below?

Spoiler alert, all but one of the species portrayed are locally indigenous to the Melbourne Metropolitan Area and the four pictures were taken there within the last year.



Top left: A sweat bee (genus *Lasioglossum*) on a wattle (genus *Acacia*) at the George Street Reserve, Sandringham, City of Bayside, Victoria. Top right: The African carderbee (*Pseudoanthidium repetitum*) on the Cut-leaved Daisy (*Brachyscome multifida*) at Sheils Reserve, West Brunswick, City of Moreland, Victoria. Bottom left: A carrot wasp (genus *Gasteruption*) on the Twiggy Daisy-bush (*Olearia ramulosa*) at Greenlink Box Hill Indigenous Nursery, Box Hill North, City of Whitehorse, Victoria. Bottom right: A pony ant (genus *Rhytidoponera*) on a tea tree (genus *Leptospermum*) at Long Hollow Heathland, Beaumaris, City of Bayside, Victoria. All images provided by the author.

Have you had a chance to look at the pictures already? Would you mind sharing which themes drew your attention the most while you are observing the images?

Did they bring about ideas of the unique traits of Australian plant species?

Or about the dichotomy between indigenous vs non-indigenous species?

Or were your thoughts drawn to think about pollination?

Were you thinking about complex ecological interactions?

Or was your focus pulled to the colours, composition, or other aesthetic elements in the images?

In the context of Australia, perhaps the images elicited a sense of Indigenous culture?

Nowadays, when I look at these images, the latter aspect is the one that I feel most strongly. But this was not always the case. Thanks to recent professional interactions with Jade Kennedy, Maddison Miller, Zena Cumpston and other Indigenous scholars with whom I have had the pleasure and luck to work with, I have come to recognise and appreciate that all Australian indigenous species – in the context of a given community associated with a given territory – have cultural Indigenous significance. I treasure this knowledge, as now every time I appreciate an Australian indigenous plant it reminds me that I am also appreciating a vital aspect of the one or more Indigenous communities to which that given plant is associated with. We might not have the fortune to interact with the Traditional Owners of the lands and waters where we live and work in our day to day lives, but it is great to realise that an integral part of their culture is reflected back to us each time we have a look at locally indigenous plants (or any other locally indigenous species for that matter).

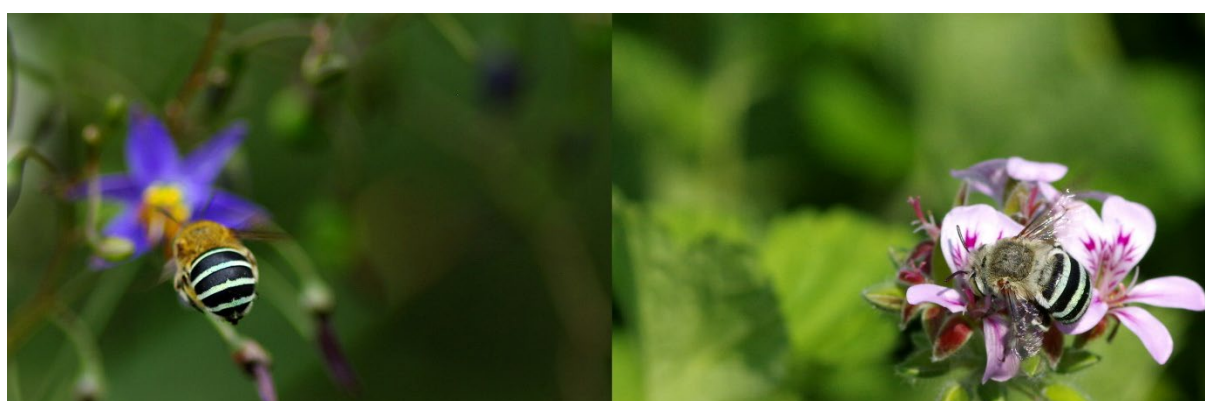
If this idea sparks your interest, I invite you to have a look at Zena Cumpston's 'Indigenous Plant Use' booklet (Cumpston 2020) and an article entitled 'Bringing nature back into cities' (Mata *et al.* 2020). The latter is an opinion piece I developed in close collaboration with Jade Kennedy, Maddison Miller, Zena Cumpston, and other colleagues from the Clean Air and Urban Landscapes Hub. The 'Australian pan-Aboriginal world views' section is of particular interest and draws extensively on Zena's and Jade's perspectives and knowledges.

Another thing I tend to do as I encounter an insect – whether in real life or photographically – is wonder if the species is locally indigenous or is introduced, exotic, alien, invasive, or otherwise non-indigenous to the area where I am seeing it (or where the picture was taken). In the Australian context, this is of course a pre-requisite to establish the bonds with Indigenous culture that I have just highlighted.

Were you able to single out the African carderbee as the sole non-indigenous species to Melbourne amongst those portrayed in the figure above? And did you noticed that she was interacting with a native Cut-leaved Daisy? In Melbourne, Australia! I am fascinated by this type of plant-insect interaction where a non-

indigenous insect species adapts to benefit from resources provided by indigenous plant species. More generally, I am intrigued by the idea that some insect species can use resources from many, if not all, the plant species they encounter, while others are quite specialised in the interactions they establish.

Take, for example, blue-banded bees – one of my favourite Australian insects – which in Greater Melbourne are represented by two species: *Amegilla asserta* and *Amegilla chlorocyanea*. Both species, and in general all blue-banded bees across Australia, are very charismatic and strikingly beautiful (see figure below). I saw my first blue-banded bee only about 5 years ago – literally the one seen in the left panel. It was visiting a patch of Black-anther Flax-lily. My thinking at the time was that blue-banded bees were flax-lily specialists and would not interact with the flowers of other plant species.



Left: A blue-banded bee (*Amegilla asserta*) flying towards a patch of Black-anther Flax-lily (*Dianella revoluta*) at Royal Park, Parkville, City of Melbourne, Victoria. Right: A blue-banded bee (*A. chlorocyanea*) on Austral Stork's-bill (*Pelargonium australe*) at Westgate Park, Port Melbourne, City of Melbourne, Victoria. Both images provided by the author.

It turns out that blue-banded bees are indeed very selective in the species they visit and are attracted to only a few other indigenous plants. As I understand, in the Melbourne Metropolitan Area, *A. asserta* and *A. chlorocyanea* are only attracted to flax-lilies (genus *Dianella*), Hop Goodenia (*Goodenia ovata*), Showy Isotome (*Isotoma axillaris*), Small Crowea (*Crowea exalata*), Bulbine Lily (*Bulbine bulbosa*), and bluebells (genus *Wahlenbergia*). If you live in Melbourne, these plants can help you attract blue-banded bees to your garden and, most importantly, support them by providing floral resources throughout the year.

Blue-banded bees, as well as many of our indigenous bees and butterflies across Australia, are also attracted to the flowers of non-indigenous plant species. In Melbourne, I have observed them on Australian native plants that are not indigenous to Victoria. These include species of emu-bush (genus *Eremophila*) and rice-flower (Genus *Pimelea*). I have also observed them or seen photographs of them on plants that are non-indigenous to Australia, including Purple-top Verbena (*Verbena bonariensis*), Chinese Plumbago (*Ceratostigma willmottianum*), Spider Plant (*Chlorophytum comosum*), tomato (*Solanum lycopersicum*), and several species of sage (genus *Salvia*). We do not fully understand if the novel resources provided by non-indigenous plants to

indigenous insects are beneficial or if, on the contrary, they may cause risks that we are not currently aware of (Valentine *et al.* 2020).

The Little Things that Run the City

The ecological interactions established between blue-banded bees and flax-lilies, Hop Goodenia, Showy Isotome, Small Crowea, Bulbine Lily, and bluebells highlight the exciting possibility of using indigenous plant species to bring indigenous nature back into our cities and towns (Mata *et al.* 2020). But what about ants, leafcutter and masked bees, ladybugs, hoverflies, assassin and damsel bugs, and parasitoid wasps, amongst many other beneficial insect groups? Can they be attracted to our parks and gardens with indigenous plants?

My colleagues and I first explored this question in 'The Little Things that Run the City' (Mata *et al.* 2016), a research project we did in the City of Melbourne across 15 public parks. In the study, we recorded the interactions between over 550 insect species – 97% were indigenous to Melbourne – and over 130 plant species (a mix of species indigenous to Melbourne, indigenous to Australia but not to Victoria, and non-indigenous to Australia), including forbs, lilioids, graminoids (both lawn and tussock species), shrubs, and trees.

The plant group associated with the largest number of insect species were neither lawns (no surprises here) nor trees (quite unexpected). As it happened, it was indigenous graminoids, a group represented by five species of tussock grasses. The champion amongst these was Common Tussock-grass (*Poa labillardierei*), on which we documented over 100 indigenous insect species. On the other side of the spectrum, lawns were associated with less than 10 insect species. We hope our findings – which we recently reported in an article entitled 'Indigenous plants promote insect biodiversity in urban greenspaces' (Mata *et al.* 2021) – will encourage architects, engineers, developers, planners, designers, and other built-environment professionals, to incorporate into their practice indigenous plant palettes that foster a larger presence of indigenous insects in our parks and gardens.

Wildlife gardening

Speaking of gardens, I would like to dedicate some words to wildlife gardening. While I have been unknowingly doing 'wildlife gardening' for many years now, I have had the good fortune to be introduced to wildlife gardening research through my colleague Laura Mumaw. Last year, we joined forces to write an opinion article in which we reviewed the wellbeing benefits of wildlife gardening and outlined how positive ecological outcomes may be reached by providing new, and improving existing, habitat for biodiversity in gardens (Mumaw and Mata 2021).

In the piece, we argue that wildlife gardening is an integrated ethic and practice to simultaneously care for one's human and ecological community. As part of a follow-up component of this collaboration (funded by Gardens for Wildlife Victoria), we have documented over 800 interactions between over 30 native midstorey plant species (forbs, climbers, shrubs, and groundcovers) and over 40 insect pollinators and other flower-visiting insects (from bees, butterflies, and hoverflies to ants, wasps, and beetles). The plants we selected for surveying were representative of those offered to Melbournian gardeners by indigenous nurseries affiliated with the Gardens for Wildlife Victoria network.

We will be making our findings available via the *Gardens for Wildlife Victoria* website soon. The information provided will include a summary of the indigenous plant species that were associated with the largest number of indigenous pollinator and flower-visiting insects, but also a detailed account of the interactions established between each plant and insect species. Whether you consider yourself to be a traditional or a wildlife gardener, we hope you may find this knowledge useful and that it will assist you in making informed decisions about which indigenous species you could plant to support local indigenous insect pollinators and other flower-visiting insects in your garden.

Acknowledgements

The author acknowledges the Traditional Custodians of the land and waterways on which the projects described in this article took place, the Wurundjeri and Bunurong people of the Kulin Nations. I pay my respects to their Elders, past, present, and emerging, and honour their deep spiritual, cultural, and customary connections to the land on which I work and live. I would like to extend my heartfelt thanks to Zena Cumpston, Maddison Miller, and Jade Kennedy for sharing with me their passion and knowledge of Australian Indigenous culture and for helping me recognise and appreciate the cultural significance of all Australian indigenous plant species. I would also like to sincerely thank Laura Mumaw for inviting me to be part of her wildlife gardening journey and to Tina Bell for kindly inviting me to write this article. Special thanks to Ken Walker, Steve Sinclair, and members of the iNaturalist community for providing identifications to some of the plant and insect species that illustrate this article.

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AFF Funded Project: New approaches for the conservation: genomics of the genus *Cycas* L. in Australia

James Clugston, Murray Henwood and Nathalie Nagalingum
Royal Botanic Gardens and Domain Trust, Sydney, NSW

Project summary*

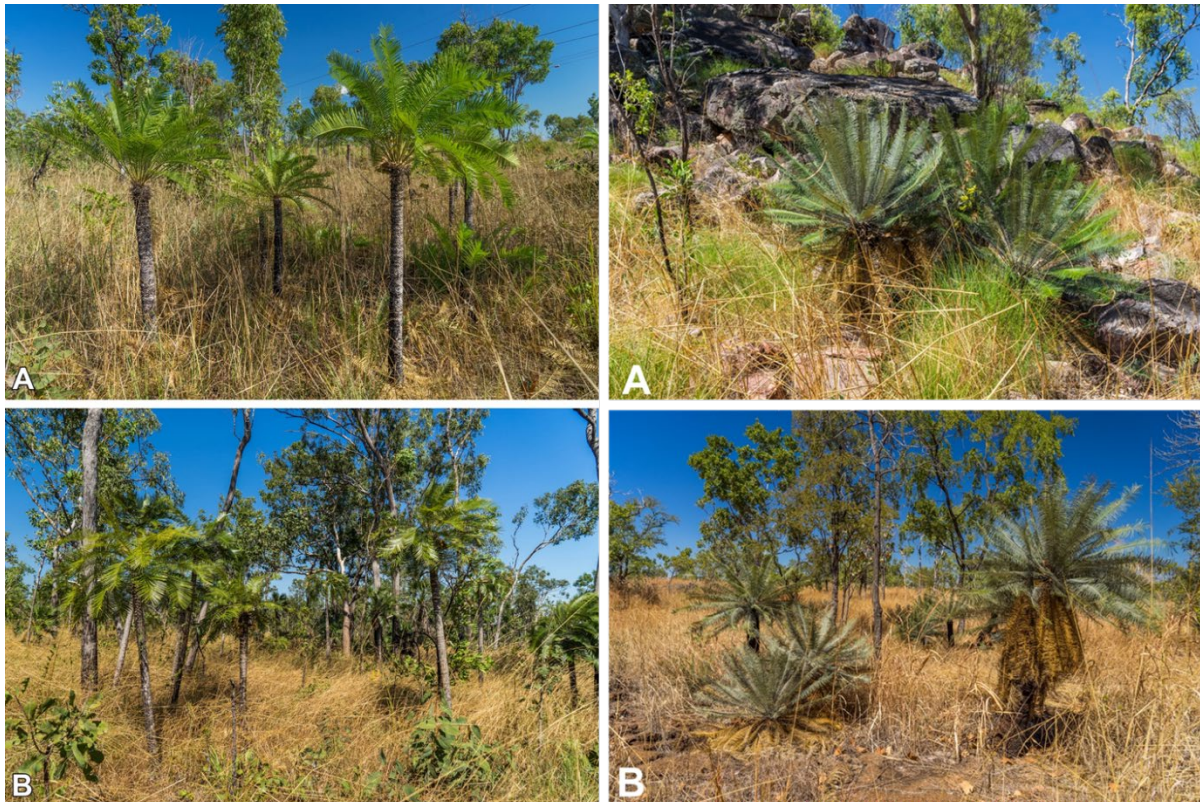
More than 60% of *Cycad* species are threatened with extinction with many existing in small and isolated populations. As a result, understanding their diversity is imperative for their conservation to ensure their long-term survival. To understand the diversity of cycads, genetics plays a fundamental role in helping us to identify how populations differ from one another. Australia represents a diversity hotspot for cycads where there are many different species and many large, clustered, and undisturbed populations. This project used the latest DNA sequencing technologies to understand the genetic diversity of populations of selected cycad species from the Northern Territory in Australia, namely: *Cycas armstrongii*, *C. calcicola*, *C. maconochiei* subsp. *maconochiei* and a hybrid population *C. armstrongii* x *C. maconochiei*.

Methods

DNA was extracted and sequenced from leaflets from a total of 60 individuals of *C. calcicola* collected from three wild populations within Litchfield National Park and three populations from the Katherine region in the Northern Territory. Individuals of varying age (juvenile to mature) and bearing microsporangiate or megasporangiate strobili were sampled. Additional samples were obtained from cultivated *ex-situ* collections in Australia and the United States. In addition, basic population demographics were recorded for each population.

For *C. armstrongii* and *C. maconochiei* subsp. *maconochiei*, DNA was extracted and sequenced from leaflets from up to 40 individuals (average = 12) collected from 21 wild populations. A population of suspected hybrid individuals (*C. armstrongii* x *C. maconochiei*) was also sampled and analysed.

Descriptive statistics included the number of individuals in each population, effective number of alleles, the effective number of alleles per locus, observed heterozygosity, expected heterozygosity, unbiased expected heterozygosity, and fixation index. The structure of the populations and genetic relationships among populations were determined based on data collected.



Top and bottom left: Populations of *Cycas armstrongii* growing in the Darwin Region (top), and near Litchfield National Park (bottom). Top and bottom right: Part of a large population of *Cycas calicicola* growing on sandstone in Litchfield National Park (top) and small population growing on limestone in the Katherine region. Images originally presented as Figures 1 and 2 in the AFF Final Report.

Results and discussion

Our results found that populations of *C. calicicola* showed evidence of inbreeding and low genetic diversity which correlated with geographic distance between major regions. The geographic disjunction between populations in the Katherine and Litchfield National Park regions were also confirmed to be disjunct based on genetic evidence. The results also showed that genetic diversity of *C. calicicola* was not well represented in botanic garden collections, presenting conservation concerns. *Cycas armstrongii* and *C. maconochiei* subsp. *maconochiei* populations also showed low levels of genetic diversity, but less inbreeding when compared to *C. calicicola*. In addition, based on DNA evidence, we found no genetic difference between *C. armstrongii* and *C. maconochiei* subsp. *maconochiei*, despite looking very different. Furthermore, these findings show that the notion of a hybrid between the two species (*C. armstrongii* x *C. maconochiei*) is invalid and that these populations can be assigned to single species.

Our results will have far-reaching significance for the conservation of vulnerable populations of cycads. In the case of *C. calicicola*, a far more structured acquisition of seeds from the wild will be required so that the species can be better preserved in botanic gardens.



Left: Large populayion of *Cycas maconochiei* subsp. *maconochiei* growing in the Cox Peninsula, Northern Territory. Right: Mature female specimen bearing many seeds on megasporophylls. Images originally presented as Figure 3 in the AFF Final Report.

Publications

This research formed part of the thesis submitted for successful completion of a PhD degree awarded by the University of Edinburgh in October 2019. One of three planned papers from this project has been published in *Molecular Ecology Resources*.

Clugston JAR, Kenicer GJ, Milne R, Overcast I, Wilson TC, Nagalibgum NS (2019) RADseq as a valuable tool for plants with large genomes – a case study in cycads. *Molecular Ecology Resources* doi: 10.1111/1755-0998.13085

*The information provided here is from a summary provided by the authors and extracted from the final report. The full report was provided to the AFF in fulfilment of the conditions of a grant awarded to the authors and can be found at <http://aff.org.au/results/grant-summaries/>.

AFF Funded Project: Seeds, seed banks and cultivation of endangered coastal salt marsh plants under climate change

Todd Minchinton

Centre for Sustainable Ecosystem Solutions and School of Earth, Atmospheric and Life Sciences, University of Wollongong, NSW

Project summary*

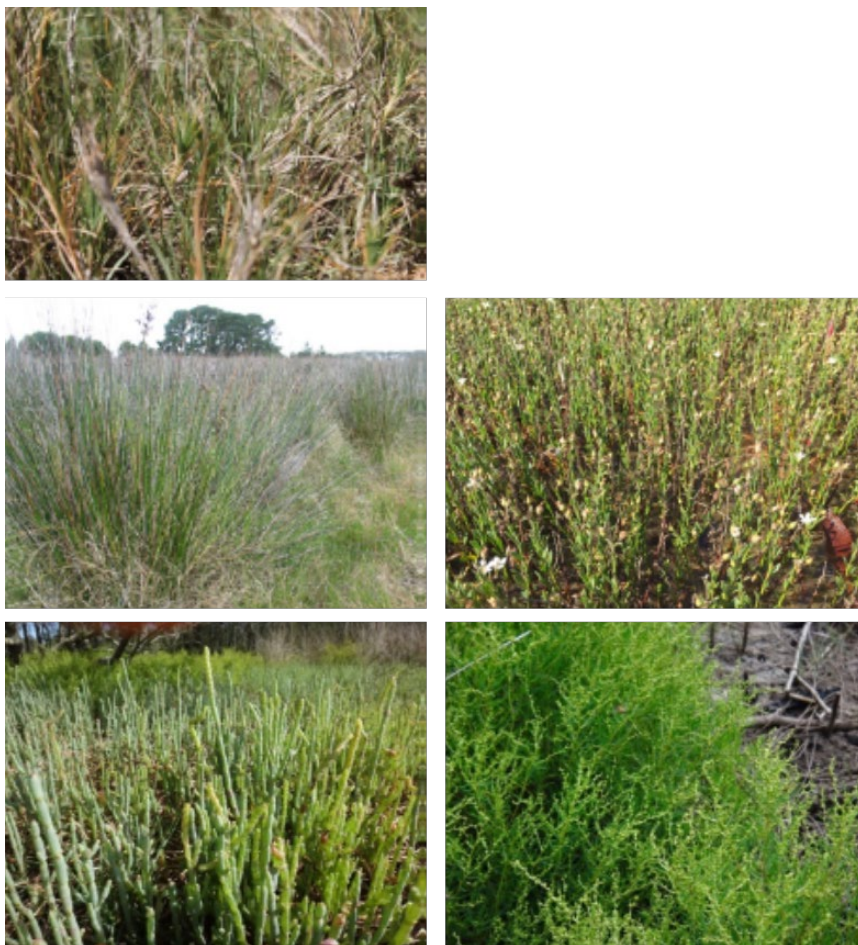
Coastal saltmarsh is an intertidal estuarine community comprised of plant species (succulent herbs, grasses, rushes, sedges, reeds, and shrubs) adapted to inundation by the tides and harsh environmental conditions in the air, water, and soil (e.g., temperature, salinity, oxygen availability). Coastal saltmarsh is recognised in Australia and globally as an important ecological community, providing habitat and food for ecologically and economically important species (e.g., fish, shellfish), acting as a natural buffer and pollution filter, and storing carbon.

Despite its ecological significance, coastal saltmarsh is one of the most highly impacted and threatened communities in NSW (with estimated losses across

estuaries in NSW since 1950 ranging from 25 to 80%) and this has led the New South Wales government to list Coastal Salt Marsh as an Endangered Ecological Community (under the NSW Biodiversity Conservation Act 2016) and to protect Marine Vegetation, including saltmarsh plant species examined here (under the NSW Fisheries Management Act 1994). Moreover, the Commonwealth government has listed Subtropical and Temperate Coastal Saltmarsh as a Vulnerable Ecological Community (under the Environment Protection and Biodiversity Conservation Act 1999).

Despite recognition of the ecological importance of coastal saltmarsh, we understand little about the resilience of the plant species that constitute the community, including their potential for natural regeneration through the production of viable seed following disturbance under varying environmental and climatic conditions or how to germinate and grow seed for restoration now and into the future.

The outcomes of this research will help to identify and cultivate key plant species for restoration of coastal saltmarshes in NSW. More broadly, this research will inform climate change adaptation and conservation strategies for important plant species in endangered coastal saltmarsh communities.



Typical dominant species found in a coastal saltmarsh ecosystem. Top left: Saltwater Couch or Sand Couch (*Sporobolus virginicus*); middle left: Sea Rush (*Juncus kraussii*); middle right: Creeping Brookweed (*Samolus repens*); bottom left: Samphire or Glasswort (*Sarcocornia quinqueflora*); bottom right: Seablite (*Suaeda australis*). Images modified from original presentation as Figure 2.3 in the AFF Final Report.

Objectives and approach

The overall objective of our research was to redress this deficiency of knowledge by examining the natural reproductive capacity, seed viability, and seed germination requirements for some of the dominant plants that constitute saltmarsh communities along the south and central coasts of NSW. An important and novel feature of our research was the examination of how variable salinity and temperature regimes, including extreme heat events, influence the viability and germination of seeds of these plant species.

Methods

We used field surveys and temperature-controlled cabinet experiments with a suite of the dominant plant species (the chenopod *Sarcocornia quinqueflora* – Samphire or Glasswort; the chenopod *Suaeda australis* – Seablite; the grass *Sporobolus virginicus* – Saltwater Couch or Sand Couch; the rush *Juncus kraussii* – Sea Rush; and the herb *Samolus repens* – Creeping Brookweed) from saltmarshes in Lake Macquarie, Brisbane Water and Jervis Bay in NSW. This comparative approach across species and estuaries was advantageous because it allowed the identification of local influences while permitting general conclusions to be made across the region.

Significant findings, implications, and practical application of the research

All coastal saltmarsh plant species examined produced viable seed and likely confers some 'resilience' to these species if aboveground vegetation is damaged. Seed is typically dispersed from adult plants into the local soil seed bank (or transported to other saltmarshes), which should lead to the production of new plants and persistence of these species. Little is known about the seed bank or seed dispersal of saltmarsh plants in Australia, and such investigations were beyond the scope of this project.

All species examined differed in their germination success and in their response to temperature and salinity. This demonstrated that species-specific differences and variations in temperature and salinity play important roles in the germination of four common coastal saltmarsh species. Such species-specific responses indicate that managing these plant species under varying environmental conditions will need to be considered on a species-by-species basis. Germination responses of seed to temperature and salinity were, however, largely in line with the apparent tolerances of adult plants and their associated distribution in the field.

Plant cover, reproductive output and seed viability for the dominant plant species, the chenopod *Sarcocornia quinqueflora* (Samphire) were generally high across all local sites and largely comparable among regions, although plants at Lake Macquarie and Brisbane Water saltmarshes were generally shorter than those at Jervis Bay and thus had less volume. The measurement of these traits is one of only a few such quantitative assessments for any saltmarsh plant in NSW, and thus provides baseline data for future comparisons.

Publications

The research has been presented in two Honours theses in the School of Biological Sciences (now School of Earth, Atmospheric and Life Sciences) at the University of Wollongong. Some of the data will be included in manuscripts that

are being prepared for publication in peer-reviewed international scientific journals.

Atton IL (2017) Reproductive biology of saltmarsh chenopods *Sarcocornia quinqueflora* and *Suaeda australis*: multiple pollination mechanisms and germination success. Honours Thesis, School of Biological Sciences, University of Wollongong, New South Wales, Australia. (co-supervised by Dr Karen Sommerville, The Australian Botanic Garden Mount Annan and Dr Amy-Marie Gilpin, Western Sydney University).

Leahy KJ (2017) Factors influencing the recruitment of coastal saltmarsh plants. Honours Thesis, School of Biological Sciences, University of Wollongong, New South Wales, Australia. (co-supervised by Dr Karen Sommerville, The Australian Botanic Garden Mount Annan and Dr Amy-Marie Gilpin, Western Sydney University).

*The information provided here was summarised from the Executive Summary of the AFF report. The full report was provided to the AFF in fulfilment of the conditions of a grant awarded to the authors and can be found at: <http://aff.org.au/results/grant-summaries/>.

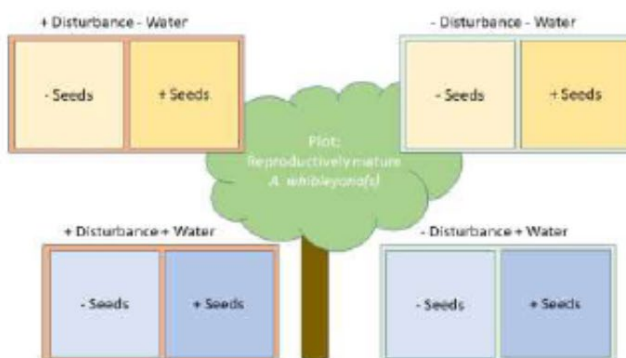
Progress Reports for AFF-Funded Projects

Soil disturbance trials to improve germination and seedling establishment for the Endangered Whibley Wattle (*Acacia whibleyana*)

Jasmin Packer, Renate Faast and Geraldine Turner
The University of Adelaide and Eyre Peninsula Landscape Board and Department for Environment and Water

Whibley Wattle (*Acacia whibleyana*) is recognised as a nationally endangered species and is one of Australia's 30 Priority Plant Species. This large shrub is restricted to a small area around Tumby Bay on the Eyre Peninsula in South Australia. One of the greatest threats to wild subpopulations of Whibley Wattle is the lack of recruitment due to altered disturbance regimes. Our experimental trials represent a partnership between the Australian Flora Foundation, the Eyre Peninsula Landscape Board, and The University of Adelaide. By bringing together land managers with research scientists, we aim to discover the influence of mechanical soil disturbance, seed supplementation, and water availability on germination and seedling establishment of Whibley Wattle.

Individual Whibley shrubs (n = 25) were established as plots for three experiments. Each plot has four subplots and eight quadrats for Experiment 1 (\pm soil disturbance with rotary hoe, \pm watering to long-term monthly average), Experiment 2 (\pm water, \pm disturbance) and Experiment 3 (\pm seed supplementation, \pm disturbance, and \pm water). Our disturbance trial was planned to begin in mid-April 2021 after breaking Autumn rains but, due to Covid-19 travel restrictions, was delayed until late June 2020. This involved application of treatments of soil disturbance and seed supplementation, then monthly watering to meet long-term monthly average, if required. All 25 plots were monitored monthly until 31 December 2020.



Left: A beautiful show of flowers of Whibley Wattle (*Acacia whibleyana*). Image provided by Jasmine Packer. Right: Experimental design of one plot with four subplots and eight quadrats for Experiment 1 (\pm disturbance, \pm water), Experiment 2 (\pm water, \pm disturbance) and Experiment 3 (\pm seeds, \pm disturbance and \pm water). Total plots = 25; total quadrats = 200.

Preliminary results include:

- Only three seeds germinated pre-disturbance (natural germination) – two were still alive in February 2021
- Almost 50 seeds germinated post-disturbance – but none were alive in January 2021
- Observations of morphology of Whibley Wattle, phenology (e.g., seed set), and health (e.g., gall phenology and dieback status)
- Increasing expertise and confidence of land managers in strategic, experimental monitoring

We are continuing to monitor the natural germination from 2020 and are watching for further post-treatment germination after the Autumn rains in 2021.



Left: Geraldine Turner and Renate Faast from The University of Adelaide applying the rotary hoe treatment. Right: Chocolate skittles in place for the rotary hoe test to determine if the skittles (and therefore Whibley seeds when applied) stay within the quadrat after rotary hoe disturbance. The answer is yes! Both images provided by Jasmin Packer.

Assessing the diversity and conservation of central Queensland rainforest using DNA barcoding

Alison Shapcott

Genecology Research Centre, School of Science and Engineering, University of the Sunshine Coast, Queensland

This project aims to add approximately 600 new plant species collected from rainforests in central Queensland to extend the existing Australian Rainforest DNA barcode reference library developed by Shapcott *et al.* (south eastern Queensland) and Crayn *et al.* (northern Queensland). The combined dataset will be used to create the most complete Australian rainforest phylogeny, based on DNA barcode markers, to date. The data will be used in conjunction with regional and community species lists to:

1. Compare the diversity of central Queensland rainforest to other regions
2. Assess and compare the distribution of rainforest diversity and distinctiveness within central Queensland to identify the areas of highest biodiversity value
3. Assess the extent and protection of central Queensland rainforests and identify priority areas for inclusion in the National Reserve System Protected Area estate
4. Determine impacts of climate change on rainforest extent to identify areas most likely to persist and that have the highest diversity and or distinctiveness

We have done most of the field work to finalise the voucher collections (as traditional herbarium samples and as digitised replicas). We now have approximately 80% of all rainforest taxa occurring in the study region as listed by the Queensland Herbarium. Voucher specimens have been lodged in the Queensland Herbarium. With cooperation of local botanists, we are planning to obtain additional specimens with the aim of obtaining greater than 85% of listed taxa.

We have extracted DNA and sequenced individual samples from the species collected for three DNA barcode markers, and we are expecting the fourth and fifth markers to be completed soon. We anticipate the final DNA barcode marker will be completed shortly after the final field collections are finalised. Thus, we are aiming to sequence at least 576 new species to add to the sequences already existing for Queensland rainforest plants.

In addition, we have accumulated more than 300 site-specific species lists from fixed plots to be used for the subsequent data analysis to supplement herbarium records for the study region. As with the remaining plant sampling, we expect to increase the number of site-specific species lists through collaborations with local botanists.

AFF Snapshot: Lasting effects of a bushfire

Ian Cox, AFF Secretary and Councillor

On Australia Day in 1975 an intense bushfire swept through parts of Castle Hill, Kenthurst and Glenorie. I know this because we had just bought our block of land at Castle Hill, and it got singed. Fortunately, we hadn't started to build the house.

The fire was helped by a strong, hot, and dry westerly wind. Where I now live at Kenthurst, the bush was burnt by this very hot fire, and only a few tree trunks were left standing. Several homes at Kenthurst were destroyed.

Scars from this long-ago fire can still be found in the bush.



Top left: This majestic eucalypt looks normal, viewed from the north. Top right: But it's not. Here's the same tree from the south. You can see the hollowed-out trunk caused by the 1975 fire. The tree would have been fairly large then and may now be up to 100 years old. Bottom: This nearby tree had a large branch blown off in a severe wind recently. The branch was weakened by the trunk damaged by the 1975 fire. The eucalypts pictured above are hybrids between *Eucalyptus racemosa* and *E. haemastoma*. All images provided by the author.



Left: This large Turpentine (*Syncarpia glomulifera*) is close to the walking trail starting at the end of Jones Road Kenthurst. As you walk past this tree it looks normal. If you have a closer look, you'll find it's anything but normal. Middle and right: The trunk, hollow to a height of 3 m, is divided into two from the base, and one side is split again. Above the 3-m hollow, the now-two trunks have healed themselves into normal/whole trunks, giving no hint of the drama below. All images provided by the author.

This abnormality in growth was caused by fire, probably more than one.



Above: More fire-damaged trees from the fire trail at the end of Porters Road Kenthurst. All images provided by the author.

All trees pictured in this story caught fire near the ground. The fire then funnelled up inside the trunk. This is one reason why it is dangerous for Rural Fire Service firefighters when they patrol the bush just after a fire has gone through an area.

The fire often does not kill the tree. It partitions off the damage and grows around it. Sometimes nature is good at repairing itself!

Acknowledgements

Sincere thanks to Lesley Waite for her help with this article.

What Research Projects Were We Funding 25 Years Ago?

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

Seed biology of Australian euphorbs

Duncan Mackay and Molly Whalen

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Funded for 2 years (1995 and 1996) for \$5,600

Summary

Many species in the family Euphorbiaceae have seeds that bear an appendage called a caruncle. Although carunculate seeds have a sporadic taxonomic distribution, some genera contain both carunculate and ecarunculate species (Webster 1994). The euphorb caruncle is often assumed to function as an elaiosome, that is, as an appendage that facilitates seed dispersal by ants, or myrmecochory. At the time, this assumption has received very little quantitative or experimental verification.

This study investigated whether carunculate seeds of species in the genus *Adriana* are dispersed by ants and if the caruncle is an attractant to seed-dispersing ants. Choice trials done in the field and laboratory indicated that the detached caruncle of *Adriana quadripartita* is indeed attractive to ants and that diaspores (seeds with the caruncle intact) are particularly attractive to omnivorous ants. Species of ants in the genus *Iridomyrmex* are generalist omnivores whereas species of *Rhytidoponera* frequently prey on other invertebrates and species of *Melophorus* often include seeds as an important component of their diets.

It has been hypothesised that elaiosomes have evolved to resemble arthropods in their fatty acid composition (Hughes *et al.* 1994). To this end, the fatty acid composition of blowflies, and seed and caruncles of species of *Adriana* were compared. The composition of blowflies resembled caruncles of *Adriana* by having relatively low concentrations of linoleic acid (10%) and intermediate concentrations of palmitic acid (18%). In contrast, seeds had relatively high concentrations of linoleic acid (62%) and low concentrations of palmitic acid (5%).

The caruncle:seed mass ratio has been shown to be an important cue eliciting diaspore removal (Mark and Olesen 1996). Measurement of diaspore and caruncle weight of seed of *Adriana quadripartita* revealed that, while there was significant variation in seed weight, the relationship between seed weight and caruncle weight did not vary significantly among populations.

While there has been steady interest in the chemical composition of ant-attracting structures on seeds in studies internationally (e.g., Fischer *et al.* 2008; Salmakis *et al.* 2011; Boieiro *et al.* 2012; Chen *et al.* 2016; Miller *et al.* 2020), there has been little additional research in Australia, particularly for the specialised structures associated with euphorbs. However, the awardees of this grant have continued with ecological seed research and have produced many publications likely to have been initiated by this study (e.g., Mackay and Whalen

1996; 1998; Whalen and Mackay 2007; Beaumont *et al.* 2009; 2013; 2016; 2017).



Left: Bitter Bush (*Adriana quadripartita*). Image from <https://gcln.org.au/product/adriana-quadrupartita>. Seed and caruncle of Leafy Spurge (*Euphorbia esula*). Image from <https://www.invasive.org/browse/detail.cfm?imgnum=5376447>.

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Significance of floral odours to animal pollinators

Anthony Oldfield
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Canberra, ACT
Funded in 1995 for \$3,000

Summary

This project investigated the olfactory preferences of three Australian Megachiropteran (bat) species, Grey-headed flying fox (*Pteropus poliocephalus*), Little red flying fox (*P. scapulatus*) and Black flying fox (*P. alecto*), for a variety of plant-derived odours.

Methods were devised and tested to investigate odour preference behaviour. This involved equipment design, development of new applications for existing statistical techniques for the analysis of preference data, and analysis of odorous headspace in test mixtures and from flowers in the field.

Preliminary behavioural observations indicated that all three bat species exhibited positive approach behaviours in response to fruit-derived odours. They responded to different quantities of test odour, with the parameters of 0.5 mL of test odour distillate at a decision distance of 125 mm producing optimal responses in *P. poliocephalus* and *P. scapulatus*. *Pteropus alecto* responded with equal intensity to all odour quantities.

A common behavioural ethogram (a catalogue of behaviours or actions exhibited by an animal) was devised for *Pteropus* and applied to develop a more detailed analyses of preference responses. These revealed that within each species, bats expressed different degrees of preference for different odours under controlled conditions. When odour preferences within a species were ranked on a decision axis, odour pairs with low separation values were more likely to change in rank

position than those further apart. Thus, not only was each species of bat able to detect fruit odours, but they could also discriminate between different odour types.

The existence of preference intransitivities in all three bat species indicated that they employ a comparative method of odour evaluation as opposed to an absolute utility method often described for foragers with transitive preferences. Thus, for these bats, the 'value' of odours is dependent upon the context within which the odour comparisons are made. Odours with low levels of separation on the preference hierarchies were more frequently associated with preference intransistivities. Furthermore, the more generalist feeder *P. alecto* appeared to have the highest sensitivity to variation in odour concentration, suggesting that dietary specialisation does not necessarily coincide with olfactory acuity.

The three species of bats did not select odours based on absolute concentration or intensity, but on qualitative characteristics. In addition, evidence from the field suggests that rhythmical variations in emissions of floral volatiles from *Eucalyptus gummifera*, a bat-preferred flower, are correlated with bee activity, with bats working trees with significantly lower concentrations of volatiles.

The potential for the development of odour-active agents to interfere with odour preference behaviour of bats in relation to reducing bat damage is substantial. Continuation of gas chromatographic-olfactometric investigation into odours involved with high incidences of preference intransitivities is strongly recommended, as is further study of bat-flower volatile interactions.

Prior to this study, the author asserted that it was not possible to state with absolute confidence that non-echolocating Megachiropterans can detect and discriminate between food-related odours. Subsequent investigations by other research groups have shown the usefulness of gas chromatography and animal behavioural studies for determining the importance of smell for location and discrimination of food by fruit bats (e.g., Hodgkison *et al.* 2007; Zhang *et al.* 2014; Gonzalez-Terrazas *et al.* 2014; Brokaw and Smotherman 2021).

Flying foxes provide important ecological functions in forests in Australia and elsewhere in the world as pollinators (e.g., Aziz *et al.* 2017) and seed dispersal agents (e.g., Parolin *et al.* 2021). Unfortunately, human populations in cities and the country have an uneasy relationship with flying foxes (e.g., <https://www.dw.com/en/flying-foxes-australias-love-hate-relationship-with-fruit-bats/a-55949095>). People living close to roosting areas (camps) in urban areas complain of the smell and noise and fruit producers can have their crops decimated (Scenic Rim Regional Council n.d.). Methods developed in the preliminary research described in this study could potentially be used to address such conflict. To date, the highly developed sense of smell has been used with limited success to deter flying foxes from commercial orchards. For example, carbide was successful in deterring flying foxes in north Queensland until they became accustomed to the smell and oxalic acid has been proposed as a possible alternative (Queensland Government n.d.). Several other deterrent smells, including prawn paste, have been trialled to try to break up camps in Sydney (Richards 2002). Conversely, olfactory cues have been use in a novel technique for forest restoration that uses the essential oils of preferred fruit to

attract frugivorous bats to degraded areas. As they spend time in the area in search of food they may defecate and import seed (Parolin *et al.* 2021).



Left: Grey-headed flying fox (*Pteropus poliocephalus*). Image from <https://www.weekendnotes.com/flying-foxes-botanic-park-adelaide/>. Middle: Little red flying fox (*P. scapulatus*). Image from <http://littleaussiebat.com.au/flying-fox-facts/>. Right: Black flying fox (*P. alecto*). Image from <https://apps.des.qld.gov.au/species-search/details/?id=984>.

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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 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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