



## **Research Centre for Ecosystem Resilience Publications**

### Foreword

The Research Centre for Ecosystem Resilience (ReCER) at Botanic Gardens of Sydney undertakes relevant research that guides the restoration and conservation of resilient ecosystems. Our diverse range of research projects are underpinned by large-scale, technologically advanced knowledge infrastructure systems that allow us to undertake genomic research on hundreds of species at a time.

The ReCER team undertakes research and collaborates across multiple areas including genomics, landscape and conservation genetics, ecological restoration, climate modelling, flora biogeography, evolution and microbiome diversity.

The knowledge obtained from our research is disseminated in several different ways including academic papers, reports, book chapters and blog posts. Here we bring together abstracts of publications from the 2024 calendar year. ReCER staff, students or research associate authors are indicated in bold.

### Peer-reviewed publications

## Chromosome-level genome assembly of the Australian rainforest tree *Rhodamnia argentea* (Malletwood).

Stephanie Chen, Ashley Jones, Patricia Lu-Irving, Jia-Yee S Yap, Marlien van der Merwe, Jason Bragg, Richard Edwards Genome Biology and Evolution 16 (11) evae238 https://doi.org/10.1093/gbe/evae238

Myrtaceae are a large family of woody plants, including hundreds that are currently under threat from the global spread of a fungal pathogen, Austropuccinia psidii (G. Winter) Beenken, which causes myrtle rust. A reference genome for the Australian native rainforest tree Rhodamnia argentea Benth. (malletwood) was assembled from Oxford Nanopore Technologies long-reads, 10x Genomics Chromium linkedreads, and Hi-C data (N50 = 32.3 Mb and BUSCO completeness 98.0%) with 99.0% of the 347 Mb assembly anchored to 11 chromosomes (2n = 22). The R. argentea genome will inform conservation efforts for Myrtaceae species threatened by myrtle rust, against which it shows variable resistance. We observed contamination in the sequencing data, and further investigation revealed an arthropod source. This study emphasizes the importance of checking sequencing data for contamination, especially when working with nonmodel organisms. It also enhances our understanding of a tree that faces conservation challenges, contributing to broader biodiversity initiatives.

### Implications for conservation and restoration:

Reference genomes, assembled using DNA sequencing, provide a platform for the use of technologies that provide new insights into management. They enable very detailed studies of diversity, and correspondingly inform the best approaches for protecting it. In conjunction with careful experiments, genomes can help identify genes that enable adaptation to challenges, including environmental change or disease. The reference genome for malletwood will help guide establishment of collections of plants that are resistant to myrtle rust, but that are also as genetically diverse as possible. The long-term goal is to reintroduce genetically diverse myrtle rust resistant populations back into the wild.

Many of our publications are open access and can be downloaded from the links provided below. Other publications and unpublished reports can be requested from the authors.

Meet the ReCER team and subscribe to our e-Newsletter at <a href="https://www.botanicgardens.org.au/recer">https://www.botanicgardens.org.au/recer</a>



Editors: Richard Bardgett, Yvonne Buckley, Jane Catford, Andrew Hector, Amy Iler, Pierre Mariotte



Karina Guo et al's use of artificial intelligence to measure leaf size in herbarium specimens was featured on the cover of the October issue of Journal of Ecology. Using machine learning to link climate, phylogeny and leaf area in eucalypts through a 50-fold expansion of leaf trait datasets.

#### Karina Guo, William Cornwell, Jason Bragg Journal of Ecology 112, 2183-2197 https://doi.org/10.1111/1365-2745.14354

1. Leaf area varies within and between species, and previous work has linked this variation to environment and evolutionary history. However, many previous studies fail to examine both these factors and often are data-limited. 2. To address this, our study developed a new workflow using machine learning to automate the extraction of leaf area from herbarium collections of Australian eucalypts (Eucalyptus, Angophora and Corymbia). This dataset included 136,599 measurements, expanding existing data on this taxon's leaf area by roughly 50-fold. Our methods were validated using field standard metrics of accuracy, and comparisons to manual measurements both from the present study and existing datasets. 3. With this dataset for the eucalypt clade, we observed positive relationships between leaf area and mean annual temperature and precipitation similar to those reported for the global flora. However, these relationships were not consistently observed within species, potentially due to gene flow suppressing local adaptation. When we examined these relationships at different phylogenetic levels, the slope of trait-climate associations within lineages converged towards the overall eucalypt slope at shallow phylogenetic scales (5–12 MY), suggesting that effects of gene flow relax just above the species level. 4. The strengthening of traitclimate correlations at evolutionary scales just beyond the intraspecific level may represent a widespread phenomenon across various traits and taxa. Future studies can unveil these relationships with the larger sample sizes of new trait datasets generated through machine learning. 5. Synthesis. Using machine learning, researchers are able to confirm current positive global relationships between leaf area and mean annual temperature and precipitation. Additionally, they were able to take this a step further and examine how it changes across time. Here they saw that at roughly 5–12 million years ago in the phylogenetic tree, the trait-climate slope begins to show significantly less variation. Overall, the study shows the potential of machine learning in ecology, with exciting new potential findings with its use.

*Araucaria bidwillii* genomics suggest Indigenous Peoples broadened translocation practices in response to settler colonialism.

Monica Fahey, Maurizio Rossetto, Emilie Ens, Ray Kerkhove People and Nature 6 (1) 286-300

https://doi.org/10.1002/pan3.10576

1. Retracing past anthropogenic dispersal of culturally important taxa offers insights to the biogeographic history of species, as well as the history of the people who interacted with them. Bunya Pine (Araucaria bidwillii Hook.) is a culturally and spiritually significant conifer tree for several Indigenous groups in eastern Australia. Sharing the edible nuts and attending Bunya gatherings is an important way for these groups to maintain their cultural connections and it has been hypothesized that prior to European colonization, Indigenous Peoples facilitated the dispersal of Bunya Pine as part of these ancient traditions. 2. We used ethnohistorical information on the use of Bunya Pine by Indigenous Peoples to interpret genomic patterns within and between disjunct distributions of Bunya Pine. 3. We found signatures of long-term isolation within the Australian Wet Tropics (AWT) and extensive gene flow within southeast Queensland (SEQ) that does not fit models of faunal or passive dispersal. Within SEQ, we found greater population structure amongst sites known to pre-date European colonization, than when colonial-era planted sites were included in our analyses, suggesting that pre-colonial translocation was sporadic or localized rather than systematic and widespread. Increased Indigenous translocations in conjunction with plantings by European settlers appears to have erased the natural pre-colonial population structure of SEQ Bunya Pine. Our stairway plot models suggest sharp population decline of SEQ Bunya Pine in the early and late Pleistocene, though we did not find evidence that anthropogenic dispersal facilitated effective population size growth of the species in the Holocene. 4. We concluded that pre-colonial translocation of SEQ Bunya Pine was likely restricted by kinship-based custodial rights, and that when Indigenous Peoples were displaced by European settlers, translocation was intensified to maintain cultural connectivity. This study is an example of how Indigenous Australian groups adapt plant management strategies to meet socio-cultural needs and demonstrates the potential for plant genomics to supplement Indigenous Biocultural Knowledge that has been impacted by colonial dispossession.

### Indigenous dispersal of Bunya Pine expanded to maintain cultural connectivity following colonisation

Fahey, et al. (2024). Araucaria bidwillii genomics suggest Indigenous Peoples broadened translocation practices in response to settler colonialism. People and Nature, 00, 1–15.

https://doi.org/10.1002/pan3.10576



Bunya Pine is a culturally significant tree for several indigenous groups in eastern Australia.

Sharing the edible nuts and attending 'Bunya gatherings' is an important way for these groups to maintain kinship and cultural connections.



Pre-colonial

Bunya

Mtns

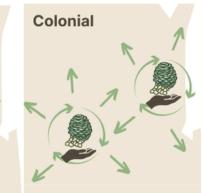
We used Bunya leaf DNA samples to infer the historical movement of Bunya across southeast Queensland. Historical references and oral knowledge on the use of Bunya by Indigenous Peoples was used to interpret the genetic data.



Blackall

Range

Queensland, Australia



Pre-colonial movement was localised or sporadic and likley restricted by kinship based cusdodial rites. Greater genetic mixing reflects increased Bunya dispersal to maintain cultural connectivity following European colonisation.

This study is an example of how Indigenous Australian groups adapt their plant management strategies to meet socio-cultural needs and demonstrates the potential for plant genomics to supplement Indigenous knowledge impacted by colonial dispossession.

Page 2

Floristic classifications and bioregionalizations are not predictors of intra-specific evolutionary patterns.

### Patrick Fahey, Richard Dimon, Marlien van der Merwe, Jason Bragg, Maurizio Rossetto

Nature Communications 15, 10770 https://doi.org/10.1038/s41467-024-54930-7

The relationship between intra-specific and inter-specific patterns and processes over evolutionary time is key to ecological investigations. We examine this relationship taking an approach of focussing on the association between vegetation and floristic classifications, summaries of inter-specific processes, and intra-specific genetic structuring. Applying an innovative, multispecies, and standardised population genomic approach, we test the relationship between vegetation mapping schemes and structuring of genetic variation across a large, environmentally heterogenous region in eastern Australia. We show that intra-specific genetic variation shows limited correspondence to vegetation and floristic classifications and is better explained by distance between sampled populations and the location of biogeographical features which limit gene flow. Mapping schemes with contiguous mapping classes, particularly larger ones, were more predictive of genetic lineages, whether based on environmental factors or not, than geographically non-contiguous schemes. We conclude that vegetation and floristic classifications are not closely correlated with intra-specific genetic patterns, showing that intraspecific processes are not recapitulated by inter-specific floristic assembly processes. This study showcases the need to implement landscape level evolutionary patterns, based on species specific datasets, in restoration and conservation activities.

### Implications and application:

That vegetation type is not predictive of genetic variation within species has important practical implications for ecological restoration and plant conservation, highlighting that while plant community type classification is a convenient way to partition floristic diversity, it does not reflect the evolutionary history or adaptive potential of single species.

A key implication for nature repair, is that when restoring a particular plant community type, constraining seed collection of a target species to a particular vegetation type is unnecessary and may reduce the long-term viability and resilience of restored vegetation.

This study highlights the value of considered seed sourcing at a landscape scale and emphasises the value in using increasingly accessible genetic tools and datasets (e.g. <u>Restore and Renew</u>) to understand landscape-level processes and maximise adaptive capacity.

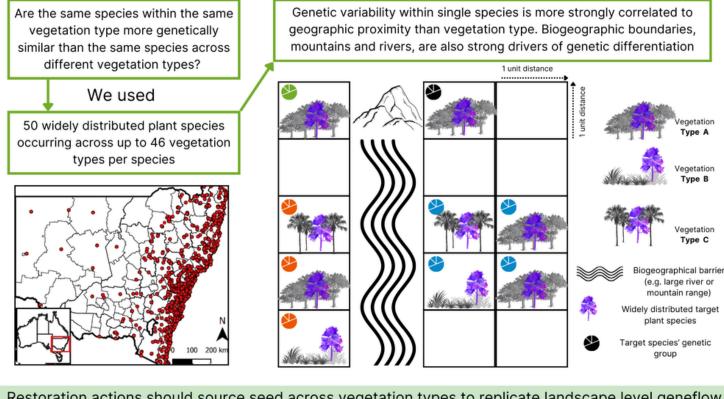


Lead Author Patrick Fahey sampling *Acacia parvipinnula* from Central Hunter Ironbark-Spotted Gum Forest, one of the 50 widespread species included in the study.

### Vegetation type is not predictive of genetic variation within plant species



We found



Restoration actions should source seed across vegetation types to replicate landscape level geneflow processes as opposed to limiting collection to discrete vegetation classification provenances. Results also suggest that species level adaptation to vegetation type is weak.

Graphical abstract. Our work showed that vegetation type is not predictive of intra species genetic diversity, rather this is driven by physical barriers and distance. The figure depicts the conceptual genetic diversity within a species at a site, denoted by piechart and purple tree, and how this varies between three adjacent or separated vegetation types (A,B,C). The landscape genetics of a mass-flowering fire-ephemeral plant.

Mira Jordan, Jason Bragg, Mark Ooi, Marlien van der Merwe, Maurizio Rossetto, Will Cornwell American Journal of Botany 111 (11) e16432 https://doi.org/10.1002/ajb2.16432

Premise: Obligate fire ephemerals are annual plants that have germination and reproduction cued by fire occurrence, persisting between fire events in a long-lived soil seed bank. Within these species, gene flow is restricted not only geographically but also temporally because individuals are limited to reproducing with others affected by the same fire event. The patchwork-like distribution of fires may therefore promote population isolation. In contrast to past fires, the Australian fires of 2019–2020 were of unprecedented extent, providing an opportunity to investigate the landscape genetics of a fire ephemeral, Actinotus forsythii, across multiple populations and to compare it to a common congener, Actinotus helianthi. Methods: For both species, we used single nucleotide polymorphisms to infer patterns of population structure and calculate measures of genetic diversity. We also estimated a phylogeny of Actinotus forsythii to understand the differentiation of a geographically isolated population. Results: For A. forsythii, the within-population diversity (allelic richness = 1.56) was greater, and the among-population differentiation (FST = 0.30) was lower than that observed for A. helianthi (allelic richness = 1.33, FST = 0.57). Actinotus forsythii had distinct geographic groupings, and a geographically isolated population of this species was genetically highly differentiated. Conclusions: Despite the fire-dependent, asynchronous gene flow, predicted between site disconnect, and possible within-site homogeneity, our results suggest that burn mosaic could be influencing gene flow patterns and fire-triggered mass flowering may promote genetic diversity within Actinotus forsythii.



### On the edge: conservation genomics of the critically endangered dwarf mountain pine *Pherosphaera fitzgeraldii*.

.....

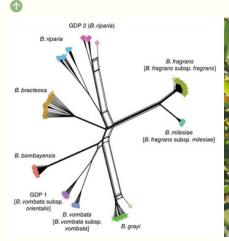
Eilish McMaster, Jia-Yee S Yap, Stephanie Chen, Ahamad Sherieff, Marianne Bate, Ian Brown, Michaela Jones, Maurizio Rossetto Basic and Applied Ecology, 80 61-67 https://doi.org/10.1016/j.baae.2024.09.003

Pherosphaera fitzgeraldii, the Dwarf Mountain Pine, is a critically endangered conifer restricted to cliff ledges within waterfall spray zones in the Greater Blue Mountains Area of New South Wales, Australia. This species is under threat from pollution, invasive weeds, and limited recruitment, which are contributing to its declining population. Using a comprehensive conservation genomic approach, we assessed the population health of P. fitzgeraldii and developed strategic recommendations for its management. Genomic analysis of P. fitzgeraldii revealed the presence of two distinct genetic groups despite the limited distribution of the species. This genetic structure aligns with prevailing wind currents, indicating potential restrictions to pollen or seed exchange between subpopulations. Furthermore, limited gene flow was observed even among nearby subpopulations, emphasizing the risk of increased genetic differentiation due to ongoing isolation. Clonality is widespread in some subpopulations, and all subpopulations displayed signs of inbreeding and genetic depletion despite subdioecy, indicative of historical constraints on gene flow and small population sizes. We offer genomic-based recommendations for prioritizing conservation sites, enhancing genetic diversity in ex situ collections, and guiding future management.

### Embracing biodiversity: multispecies population genomics of leafless Bossiaea species shows novel taxa, population dynamics and conservation strategies.

Eilish McMaster, Jia-Yee S Yap, Keith McDougall, Elizabeth James, Neville Walsh, Nic Jario, Jessica Peterie, Maurizio Rossetto Australian Systematic Botany 37, SB23031 https://doi.org/10.1071/SB23031

Leafless Bossiaea species are a unique group of plants endemic to Australia that present intriguing challenges in taxonomy and conservation because of their morphological simplicity and often small, disjunct populations. We employed whole genome reduced representation sequencing (DArTseq) to enhance our understanding of the population dynamics, diversity and taxonomy of leafless Bossiaea species. Our dataset comprised 52,539 single-nucleotide polymorphisms across 283 samples from 7 leafless Bossiaea species, including 6 listed threatened species. We examined population structure, phylogenetic relationships, kinship and gene flow within and among populations. On the basis of our population-genomic analyses, we propose recognition of the novel taxon Bossiaea vombata subsp. orientalis and a change in status from species (B. milesiae) to subspecies for B. fragrans subsp. milesiae. Additionally, we show extensive clonal reproduction across species and limited gene flow at distances of >1 km, shedding light on the challenges faced by these species. We advocate a coordinated approach to conservation, focusing on restoring self-sustaining populations and leveraging genetic rescue strategies. By addressing the population dynamics of multiple species simultaneously in taxonomically challenging lineages, we can make informed choices to safeguard biodiversity and evolutionary potential.





Working with the NSW Saving Our Species program, ReCER geneticists have be able to review the classification of seven leafless Bossiaea, five of which are threatened with extinction.

.....

Further studies to uncover the sex determination mechanism of *P*. *fitzgeraldii* could help maintain balanced sex ratios in ex situ collections and support future genetic rescue efforts. This study underscores the value of genomics in informing protection and recovery of unique threatened species.



Eilish McMaster with an ex situ collection of Pherosphaera fitzgeraldii at Blue Mountains Botanic Garden Mount Tomah, created with guidance from the genomics research.

Adaptive responses to living in stressful habitats: Do invasive and native plant populations use different strategies?

Justin Wan, Stephen Bonser, Clara Pang, Fatih Fazlioglu, Susan Rutherford Ecology Letters 27 (3) e14384 <u>https://doi.org/10.1111/ele.14419</u>

Plants inhabit stressful environments characterized by a variety of stressors, including mine sites, mountains, deserts, and high latitudes. Populations from stressful and reference (non-stressful) sites often have performance differences. However, while invasive and native species may respond differently to stressful environments, there is limited understanding of the patterns in reaction norms of populations from these sites. Here, we use phylogenetically controlled meta-analysis to assess the performance of populations under stress and non-stress conditions. We ask whether stress populations of natives and invasives differ in the magnitude of lowered performance under non-stress conditions and if they vary in the degree of performance advantage under stress. We also assessed whether these distinctions differ with stress intensity. Our findings revealed that natives not only have greater adaptive advantages but also more performance reductions than invasives. Populations from very stressful sites had more efficient adaptations, and performance costs increased with stress intensity in natives only. Overall, the results support the notion that adaptation is frequently costless. Reproductive output was most closely associated with adaptive costs and benefits. Our study characterized the adaptive strategies used by invasive and native plants under stressful conditions, thereby providing important insights into the limitations of adaptation to extreme sites.

Using molecular and morphometric data as operational criteria for the analysis of a threatened rainforest species complex shows interspecific variation, with implications for cryptic-species delimitation and conservation.

.....

Aaron Brunton, Paul Forster, Steven Ogbourne, **Maurizio Rossetto**, David Schoeman, Gabriel Conroy Australian Systematic Botany 37 SB23024 https://doi.org/10.1071/SB23024

Resolution of systematic associations and species boundaries is vital for developing conservation priorities for threatened taxa. A complex of Fontainea (Euphorbiaceae) populations, endemic to rainforest communities in central-eastern Australia, comprises several taxonomically challenging species. Fontainea oraria is Critically Endangered, with only one natural population of 10 mature individuals, with the closely related species F. australis being listed as Vulnerable. A recently discovered Fontainea population (currently nominated as F. sp. Coffs Harbour) with taxonomic similarities to F. oraria has been provisionally listed as Critically Endangered, with fewer than 40 individuals. This study employed an integrative approach, combining genetic data and morphometrics to determine species boundaries for three threatened Fontainea population groups as a model system for an integrative approach to delimiting cryptic species. Although our results suggest the potential subdivision of the population groups into three taxa, caution is warranted because this hypothesis remains inconclusive. Taxonomic challenges demand a careful approach, acknowledging the possibility of alternative interpretations. Contrary to supporting distinct species, our morphological and genetic data may also be interpreted as indicative of a single species with geographic variation, a phenomenon observed in genetically linked populations experiencing isolation by distance. Overall, we highlight the need for further research to establish species limits to guide conservation actions.

The geno-geo-climate nexus: contributions of geographic and ecological factors in shaping the genomic divergence of two closely related threatened rainforest species of Fontainea Heckel (Euphorbiaceae).

.....

Aaron Brunton, Keaka Farleigh, Steven Ogbourne, **Maurizio Rossetto**, David Schoeman, Gabriel Conroy Landscape Ecology 39 (11) <u>https://doi.org/10.1007/s10980-024-01828-w</u>

Context: Processes that shape genomic and ecological divergence can reveal important evolutionary dynamics to inform the conservation of threatened species. Fontainea is a genus of rainforest shrubs and small trees including critically endangered and threatened species restricted to narrow, but complex geographic and ecological regions. Several species of Fontainea are subject to spatially explicit conditions and experience limited intra-specific gene flow, likely generating genetic differentiation and local adaptation. Objectives: Here, we explored the genetic and ecological mechanisms underlying patterns of diversification in two, closely related threatened Fontainea species. Our aim was to compare spatial patterns of genetic variation between the vulnerable Fontainea australis (Southern Fontainea) and critically endangered F. oraria (Coastal Fontainea), endemic to the heterogeneous subtropical region of central, eastern Australia, where large-scale clearing as severely reduced rainforest habitat to a fraction (<1%) of its pre-European settlement extent. Methods: We used a set of 10,000 reducedrepresentation markers to infer genetic relationships and the drivers of spatial genetic variation across the two species. In addition, we employed a combination of univariate and multivariate genome-environment association analysis using a set of topo-climatic variables to explore potential patterns of local adaptation as a factor impacting genomic divergence. Results: Our study revealed that Coastal Fontainea have a close genetic relationship with Southern Fontainea. We showed that isolation by distance has played a key role in their genetic variation, indicating that vicariance can explain the spatial genetic distribution of the two species. Genotype-environment analyses showed a strong association with temperature and topographic features, suggesting adaptation to localised thermal environments. We used a multivariate redundancy analysis to identify a range of putatively adapted loci associated with local environmental conditions. Conclusions: Divergent selection at the local-habitat scale as a result of dispersal limitations and environmental heterogeneity (including physical barriers) are likely contributors to adaptive divergence between the two Fontainea species. Our findings have presented evidence to indicate that Southern and Coastal Fontainea were comprised of distinct genetic groups and ecotypes, that together may form a single species continuum, with further phenotype research suggested to confirm the current species boundaries. Proactive conservation actions, including assisted migration to enhance the resilience of populations lacking stress-tolerant single nucleotide polymorphisms (SNPs) may be required to secure the long-term future of both taxa. This is especially vital for the critically endangered Coastal Fontainea given projections of habitat decline for the species under future climate scenarios.

Not all edible nuts are eaten: evidence for continued Aboriginal cultural use and dispersal of Bunya Pine (*Araucaria bidwillii*) in southern but not in northern Queensland.

Patrick Cooke, Emilie Ens, Philip Allan Clarke, H Chang, **Maurizio Rossetto**, Darren Crayn, G Turpin and Asa Ferrier Journal of Ethnobiology 44(4) <u>https://doi.org/10.1177/02780771241246853</u>

Globally, there is increasing evidence that Indigenous peoples have manipulated plant and animal populations over millennia. The Bunya Pine (Araucaria bidwillii Hook.) is a coniferous tree native to subtropical and tropical eastern Australia, which produces 30-100 large, edible seeds within a large cone weighing 5-10 kgs. Endemic Bunya populations occur as two disjunct populations: one in southeastern Queensland/north-east New South Wales and the other in northern Queensland. We investigated possible pre-and postcolonial Bunya seed dispersal within and between the two separate populations by utilising multidisciplinary methods to examine Aboriginal cultural connections with Bunya. Analysis of historical sources and ethno-graphic interviews conducted with Aboriginal knowledge holders provided strong evidence for localised deliberate dispersal within the southern population but no solid evidence for dispersal in the north, suggesting two distinct modes of Aboriginal interaction with this species. Early European colonial records documented the Indigenous cultural significance of southern Bunya, evidenced by large feast gatherings, creation Stories, Aboriginal familial tree connections, seed carrying and planting, and storage in camps. The continued cultural attachment postcolonisation was evident from ethnographic interviews with Indigenous knowledge holders that revealed plantings of this species on Aboriginal missions and modern grave sites. The development of an overarching effective biocultural management plan to conserve the biological and cultural importance of this species is therefore particularly important for the southern population, especially in the face of new threats from pathogenic dieback. The individal tree connections of certain Aboriginal families also warrant deeper ethnographic study to target planting and restoration efforts that preserve these kin-based biocultural connections.

## Environmental and biogeographic drivers behind alpine plant thermal tolerance and genetic variation.

### **Lisa Danzey**, Verónica Briceño, Alicia Cook, Adrienne Nicotra, Gwendolyn Peyre, **Maurizio Rossetto**, **Jia-Yee S Yap**, Andrea Leigh Plants 2024, 13 (9)

https://doi.org/10.3390/plants13091271

In alpine ecosystems, elevation broadly functions as a steep thermal gradient, with plant communities exposed to regular fluctuations in hot and cold temperatures. These conditions lead to selective filtering, potentially contributing to species-level variation in thermal tolerance and populationlevel genetic divergence. Few studies have explored the breadth of alpine plant thermal tolerances across a thermal gradient or the underlying genetic variation thereof. We measured photosystem heat (Tcrit-hot) and cold (Tcrit-cold) thresholds of ten Australian alpine species across elevation gradients and characterised their neutral genetic variation. To reveal the biogeographical drivers of present-day genetic signatures, we also reconstructed temporal changes in habitat suitability across potential distributional ranges. We found intraspecific variation in thermal thresholds, but this was not associated with elevation, nor underpinned by genetic differentiation on a local scale. Instead, regional population differentiation and considerable homozygosity within populations may, in part, be driven by distributional contractions, long-term persistence, and migrations following habitat suitability. Our habitat suitability models suggest that cool-climate-distributed alpine plants may be threatened by a warming climate. Yet, the observed wide thermal tolerances did not reflect this vulnerability. Conservation efforts should seek to understand variations in species-level thermal tolerance across alpine microclimates.

### A genetic investigation of relationships and species boundaries between *Eucalyptus dalrympleana* and allied taxa in New South Wales.

.....

Patrick Fahey, Maurizio Rossetto, Bruhl Jeremy, Rose Andrew, Damien Andrew Telopea 27, 177–196 https://doi.org/10.7751/telopea18544

A revision of the classification of taxa in the *Eucalyptus dalrympleana* complex within *E*. series *Viminales* from New South Wales using population level genetic data is presented here. It remains unclear if the complex is a natural grouping to the exclusion of other related species, and hybridisation between members of the complex and *E. mannifera*, *E. viminalis* and *E. elliptica* is recorded. In line with observations from field surveys, the two taxa from the North Tablelands region, *E. dalrympleana* subsp. *heptantha* and *E. rubida* subsp. *barbigerorum* formed a single genetic lineage and the latter is therefore considered a synonym of the former, given this lineage is most closely related to *E. calnympleana* subsp. *dalrympleana*. The threatened narrow-range endemic taxon *E. canobolensis* is shown to be nested within the more widespread *E. rubida* subsp. *rubida*, and therefore the original circumscription of this taxon as *E. rubida* subsp. *canobolensis* is supported.

## Multinational evaluation of genetic diversity indicators for the Kunming-Montreal Global Biodiversity Framework.

.....

Mastretta-Yanes A, et al. (c. 50 authors) incl. Marlien van der Merwe Ecology Letters 27 (7) Je14461 https://doi.org/10.1111/ele.14461

Under the recently adopted Kunming-Montreal Global Biodiversity Framework, 196 Parties committed to reporting the status of genetic diversity for all species. To facilitate reporting, three genetic diversity indicators were developed, two of which focus on processes contributing to genetic diversity conservation: maintaining genetically distinct populations and ensuring populations are large enough to maintain genetic diversity. The major advantage of these indicators is that they can be estimated with or without DNA-based data. However, demonstrating their feasibility requires addressing the methodological challenges of using data gathered from diverse sources, across diverse taxonomic groups, and for countries of varying socio-economic status and biodiversity levels. Here, we assess the genetic indicators for 919 taxa, representing 5271 populations across nine countries, including megadiverse countries and developing economies. Eighty-three percent of the taxa assessed had data available to calculate at least one indicator. Our results show that although the majority of species maintain most populations, 58% of species have populations too small to maintain genetic diversity. Moreover, genetic indicator values suggest that IUCN Red List status and other initiatives fail to assess genetic status, highlighting the critical importance of genetic indicators.

The susceptibility of rare and threatened NSW species to the root-rot pathogen *Phytophthora cinnamomi*: 2. The identification of species requiring protection or further research.

### Keith McDougall, Edward Liew

Australian Journal of Botany 72, BT23106 https://doi.org/10.1071/BT23106

Context. The response of most native plant species in New South Wales (NSW) to infection by the oomycete pathogen Phytophthora cinnamomi is unknown, which makes decisions about disease management difficult. Aims. We aim to improve knowledge about the potential threat from P. cinnamomi by testing a further 32 threatened species for their response to the pathogen and developing a method for prioritising management and susceptibility testing. Methods. Susceptibility to infection and host response were evaluated in glasshouse experiments where the pathogen was introduced to pots containing the threatened species, and the results were compared with control uninoculated pots. Our prioritisation used modelled habitat suitability for P. cinnamomi, proximity to known P. cinnamomi occurrences, and numbers of plant species populations at least 1 km apart to rank 928 rare and threatened plant species native to NSW for either management or susceptibility testing. Key results. Phytophthora cinnamomi was re-isolated from the roots of 10 of the 32 species assessed, most of which also showed significant mortality or disease symptoms. Darwinia peduncularis, Hibbertia circinata, Isopogon fletcheri, Phebalium speciosum, Pultenaea baeuerlenii and Pultenaea parrisiae were the most severely affected species. The effect of P. cinnamomi is known for only 63 rare and threatened species in NSW. The Greater Sydney region is a hotspot for rare and threatened plant species with a high priority for susceptibility testing. Conclusions. The prognosis in the wild for rare and threatened plant species affected by P. cinnamomi depends on (1) habitat suitability for the pathogen, with subalpine and arid-zone species unlikely to be affected, (2) the number of unaffected populations, with two severely affected species that occur only on infested sites (Hibbertia circinata and Prostanthera marifolia) facing extinction in the near future and (3) climate, with some species (e.g. Pomaderris delicata) apparently affected only in unusually wet years. Further susceptibility testing of rare and threatened species is required. This should be supported by taxonomic studies of genera (e.g. Hibbertia, Pultenaea) commonly affected by the pathogen. Implications. Many more plant species in NSW are likely to be severely affected by *P. cinnamomi* than currently known, and may require active management of the disease for their long-term survival.



ReCER team planting a Wollemi Pine at Mount Tomah October 2024



RecER moved into their new office and laboratory facilities within the refurbished Brown Building in 2024. With the team now numbering twenty staff, it is great to be housed together in a space that encourages collaboration.

# Resilience to summer bushfire in the threatened orchid, *Caladenia tessellata*, in terms of pollination success, herbivory, and mycorrhizal associations.

### Ryan Phillips, Jude Hatley, Xia Li, **Richard Dimon**, Noushka Reiter Botanical Journal of the Linnean Society 205 (4) <u>https://doi.org/10.1093/botlinnean/boad079</u>

In some biogeographic regions, many threatened plant species occur in habitats that periodically experience bushfire. However, we currently have relatively little information on how important plant-animal and plant-fungus interactions are affected by these fires. For the threatened sexually deceptive orchid Caladenia tessellata we test whether pollination rate, frequency of florivory, and the species of mycorrhizae the plant associates with differ between burnt and unburnt sites. Interestingly, pollination rates were unaffected by fire, demonstrating that populations of the thynnine wasp pollinator can persist post-fire. However, there was a significant negative relationship between number of flowers in a population and pollination success, which is likely a by-product of a deceptive pollination strategy. Despite the presence of vertebrate herbivores, florivory rates were low in both burnt and unburnt sites. Caladenia tessellata associated primarily with the mycorrhiza Serendiptia australiana, regardless of fire history. While our results suggest resilience to a one-off summer fire for the ecological interactions that we measured, it would be interesting to investigate the effects of fire frequency and time of year. High reproductive rates in small populations of C. tessellata suggest these populations may be viable and that retaining them is a high priority for conservation.

Building meaningful collaboration in conservation genetics and genomics.

.....

#### Robyn Shaw, et al. (c. 30 authors) including **Maurizio Rossetto** Conservation Genetics 25, 1127-1145 https://doi.org/10.1007/s10592-024-01636-4

Genetic diversity is the foundation of biodiversity, and preserving it is therefore fundamental to conservation practice. However, global conservation efforts face significant challenges integrating genetic and genomic approaches into applied management and policy. As collaborative partnerships are increasingly recognized as key components of successful conservation efforts, we explore their role and relevance in the Australian context, by engaging with key entities from across the conservation sector, including academia, botanic gardens, herbaria, seed banks, governmental/non-governmental organisations, private industry, museums, Traditional Owners, Indigenous rangers, and zoos and aquaria. By combining perspectives from these entities with comprehensive literature review, we identified five guiding principles for conservation genetic and genomic research and explored the different elements of, and approaches to, collaboration. Our reflections suggest that there is a substantial overlap in research interests across the Australian conservation sector, and our findings show that collaboration is increasing. We discuss approaches to building collaborative partnerships, the reciprocal benefits of collaborating, and some remaining challenges associated with data generation, data collection, and cross-cultural considerations. We emphasise the need for long-term national resourcing for sample and data storage and consistency in collecting, generating and reporting genetic data. While informed by the Australian experience, our goal is to support researchers and practitioners to foster meaningful collaborations that achieve measurable management outcomes in conservation genetics and genomics, both in Australia and globally.



## Other publications

Walking the trees: we traced how First Nations groups moved bunya pine and black bean trees.

## Monica Fahey, Emilie Ens, Maurizio Rossetto, Oliver Costello, Patrick Cooke

The Conversation, 18th September

For millennia, Indigenous knowledge holders have passed down lore to the next generation. Much lore describes the relationships between people and Country, including custodial responsibilities to care for other species as kin. Each species carries a history of movement and change in its DNA. By quantifying how related one individual of a species is to another, evolutionary ecologists can infer how a species migrated in the deep past. When we combine both types of knowledge – lore and genetics – we can make new discoveries. Our recent body of research uses genomic techniques and interviews with First Nations knowledge holders to investigate whether First Nations Peoples moved two culturally important food sources, bunya pines and black bean trees. <u>Read more</u>

## Case study: Threatened plant translocation, *Eucalyptus cryptica*, Myrtaceae.

Enhua Lee, **Susan Rutherford**, **Jason Bragg**, **Maurizio Rossetto**, **Trevor Wilson**, and **Jia-Lee Samantha Yap** Australasian Plant Conservation 33 (1) 3-6

This case study summarises a translocation project for the critically endangered mallee *Eucalyptus cryptica*. The project aims to establish and maintain new genetically diverse population(s). The case study documents key stakeholders, site selection, translocation proposal, pre-translocation preparation, design, implementation and ongoing management, along with key learnings and outcomes. Seedlings used in the translocation captured genetic variation that is representative of the species and displayed minimal kinship to reduce the risk of inbreeding.



### New Update

## **ReCER Field Collection**

In 2024, the ReCER Field Collection app was updated to incorporate additional fields related to seed collection.

Our Field Collection App is a mobile app that enables plant field collectors to accurately record field data associated with samples using a digital device (smart phones and tablets).

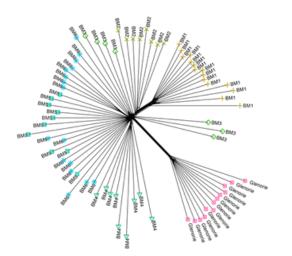
The app is freely available for other field collectors and can be downloaded from the <u>Apple App Store</u> or <u>Google Play Store</u>.

## Unpublished reports prepared for the NSW Saving our Species program

## Conservation Genomics of *Acacia gordonii* in support of management.

## Eilish McMaster, Jia-Yee Samantha Yap, Caroline Cristofolini, Maurizio Rossetto

Acacia gordonii, commonly known as Gordon's wattle, is an endangered shrub species native to the Sydney Basin Bioregion, NSW. It has a limited distribution range and faces persistent threats such as inappropriate fire regimes, habitat loss, and climate change. This study aimed to enhance the conservation management of A. gordonii. The study confirmed the species' distinction from closely related species including the similar looking A. baueri subsp. aspera, and identified A. gordonii has two subpopulations: one in the Blue Mountains and another in Glenorie, characterised by limited gene flow between them. The restricted gene flow is driven by a combination of natural isolation due to geographic distance (Glenorie and the Blue Mountains being 40km apart) and human-induced land clearing (separated by the Cumberland plain). Analysis of genetic health detected inbreeding and moderate to low diversity across populations, with the isolated Glenorie and Bells Line (BM1) sites exhibiting notably lower diversity and unique alleles than other sites. Based on these genomic findings, we recommend setting up an ex-situ collection that can contribute to future translocation projects. Guidance is provided in the form of sampling strategies that maximize genetic diversity capture in an ex-situ collection. Overall, this study provides new insights into the population dynamics of A. gordonii and offers practical actionable recommendations to support its conservation and long-term viability in the face of ongoing threats.



Genomic data revealed and identified two subpopulations of Acacia gordonii, characterised by limited gene flow between them.

.....

## Conservation genomics of *Acacia meiantha* in support of management.

### Manuela Cascini, Jia-Yee Samantha Yap, Maurizio Rossetto

Acacia meiantha, or barradam-bang wattle, is an endangered shrub distributed in four disjunct locations in the Central Tablelands - Clarence (in the Blue Mountains), Aarons Pass, Mullions Range State Forest, and near the village of Hargraves (recently discovered). The species is clonal, and recruitment is low, highlighting the precarious nature of the species. This study applied genome-wide SNP data to gain insights into the extent of clonality, the level of genetic diversity and gene flow and to provide genomically informed advice about species' conservation. The genomic analyses identified extreme clonality at Clarence with the detection of only two genets, and less extensive clonality at other subpopulations. Genetic variation is mainly characterised by the slight genetic divergence between individuals from Hargraves and other sites. This is surprising considering that Clarence is geographically the most distant subpopulation. This result, in combination with the high relatedness between individuals, and high levels of heterozygosity, suggest that individuals of Hargraves may be of hybrid origin. Based on the genomic findings of this study, we propose actions for Acacia meiantha that include establishing optimised ex-situ collections and implementing more targeted efforts (including more surveys and genetic screening) aimed at improving the health of the Clarence subpopulation and better understanding the Hargraves subpopulation.

Conservation genomics of *Eucalyptus camaldulensis* in the Hunter Catchment in support of genetically informed seed sourcing.

### Patrick Fahey, Patricia Hogbin, Marlien van der Merwe, Maurizio Rossetto

Eucalyptus camaldulensis is the most widespread eucalypt species in Australia and is an iconic part of many landscapes, however land use changes and clearing has had major negative impacts on many populations of the species. This includes the Hunter catchment that is listed as an endangered population and is the target of several conservation and restoration projects within the Hunter region. This study uses high throughput genomic data to assess the genetic relationships of the Hunter Catchment E. camaldulensis population to the species wider distribution, assess levels of genetic diversity and health of remnant stands in the region, and develop genetically informed seed sourcing strategies to maximise the success and self-sustainability of restored populations of the species in the region. It is shown that E. camaldulensis frequently hybridises with other Red Gum species where they co-occur both within and outside the Hunter catchment, and that all stands in the region are E. camaldulensis subsp. camaldulensis, with close genetic relationships to populations in the Darling River catchment, especially those in the Namoi catchment. Based upon the frequency of hybrids and levels of genetic diversity, it is recommended seeds are sourced from the largest stands of E. camaldulensis, and preferentially from stands along major waterways, rather than smaller tributaries, to minimise the collection of hybrid seed and maximise captured genetic diversity. Seedlings from seed collections by the Broke Bulga Landcare group are assessed and optimised supplementation plans are developed for both the Landcare managed restoration site and a local government managed remnant stand in Muswellbrook.



Genetic guidelines are informing the sourcing of diverse 'climate-ready' seed for restoration of River Red Gum in the Hunter Valley.

## Conservation genomics of *Euphorbia psammogeton* in support of management.

#### Manuela Cascini, Jia-Yee Samantha Yap, Maurizio Rossetto

Euphorbia psammogeton, or sand spurge, is an endangered coastal perennial herb found in QLD and NSW, and on Lord Howe Island. Once widespread along the coast, its distribution has significantly contracted, leaving only small, fragmented populations. These are under threat from competition with bitou bush and ongoing habitat disturbance, jeopardising continued survival of the species. This study applied genome-wide SNP data to assess genetic diversity, gene flow, and patterns of relatedness, aiming to provide genomically informed guidance for species management. The analyses revealed several key insights: (1) genetic diversity is distributed between rather than within sites and follows an isolation by distance pattern, indicating any new sites are expected to possess unique genetic diversity not represented by existing populations, and thus need to be protected; (2) Overall low heterozygosity levels and high relatedness suggest the species has a tendency to self, and the detection of near genetically identical individuals may be the result of inbreeding between highly similar individuals; (3) The observed clonality may also be due to the resampling of a single extended individual or apomixis, as known in other species. Based on these genomic findings, we provide several recommendations to enhance the genetic health of Euphorbia psammogeton. We determined the selection of E. psammogeton to sample in order to create genetically representative collections that can be used for new for translocation plantings or augmentation purposes. We recommend implementing a robust tagging system to ensure accurate tracking of transplanted plants and surveying unexplored areas with suitable habitat. The northern area in NSW, encompassing the Middle Bluff, North, and South Freshwater Beach sites, is more isolated than other sites and thus at higher risk of decline, making targeted augmentation efforts important for their survival.

## Conservation genomics of *Haloragodendron lucasii* in support of its long-term management.

### Caroline Cristofolini, Chantelle Doyle, Jia-Yee Samantha Yap, Maurizio Rossetto

Haloragodendron lucasii, an extremely rare species distributed on the north shore of Sydney, was presumed extinct until its rediscovery in 1986 and is currently listed as endangered. Following its discovery, a genetic study found the species to be extensively clonal and to consist of seven genets. More than 20 years on, multiple ex-situ collections have been established, and an updated study is needed to re-examine the species' diversity in-situ and determine whether the diversity within the ex-situ collections is representative. The study set up followed the published conservation genomics workflow, and included addressing whether a geographically close species, H. gibsonii, which was relatively recently described and known to resemble H. lucasii, is in fact genetically distinct from it. The study determined that H. lucasii and H. gibsonii are genetically distinct. An examination of only H. lucasii samples, collected representatively across its extant distribution and known ex-situ collections, found only seven genets, substantiating the species has minimal remaining diversity. The genets were however not the same ones identified in a previous genetic study. The results overall suggest the species once had a greater number of genets but is now in decline in-situ. To secure the species' survival, it is crucial that the diversity of H. lucasii be maintained ex-situ. Currently, none of the ex-situ collections are fully representative of the species' diversity. A fully represented collection enables an experimentally coordinated mating program be implemented to assess the species' sexual viability.



Caroline Cristofolini collecting pollen for experimental cross pollination guided by genetic research.

.....

## Conservation genomics of Homoranthus in support of management.

### Eilish McMaster, Jia-Yee Samantha Yap, Maurizio Rossetto

Homoranthus is a genus of plants in the Myrtle family Myrtaceae, including multiple species with very limited ranges and often found in only one or two locations. This project focused on analysing the genetic health of 12 Homoranthus species from the diverse region along the NSW-QLD border, involving 353 plant samples. Eleven of these species are considered threatened, and seven are managed through the Saving our Species (SoS) program. The study provides a broad analysis on Homoranthus as well as focused insights into our original target SoS species, *H. bebo* and *H. croftianus*, with sampling contributed by our collaborator, the University of New England (UNE). Our research uncovered several key findings:

- Genetic Isolation and Diversity: All Homoranthus species studied show signs
  of inbreeding and have lower genetic diversity than usual. Additionally, these
  plants similarly have limited gene flow between populations, even over short
  distances. This pattern likely contributes to the rapid formation of
  differentiated lineages in the small areas they occupy.
- **Discovery of a new lineage:** We identified a new genetically differentiated lineage near Torrington, previously thought to be a population of *H. lunatus*. This lineage and *H. lunatus* warrant taxonomic assessment.
- **Clonality:** We found that clonality is not common among these species. This is a preliminary finding that warrants further investigating as this study sampling strategy did not specifically test for clonality and focused on capturing representative diversity within populations.
- **Species-Specific Insights:** *H. bebo*, which has a larger population size, shows relatively higher genetic diversity compared to other species in the genus. Whereas *H. croftianus* shows notable genetic isolation between its sites, even though they are only a few hundred meters apart. This isolation suggests a need for interventions such as moving plants between sites or assisting their pollination to improve genetic exchange.

## Conservation genomics of *Isopogon fletcheri* in support of management.

### Manuela Cascini, Jia-Yee Samantha Yap, Philip Gleeson, Maurizio Rossetto

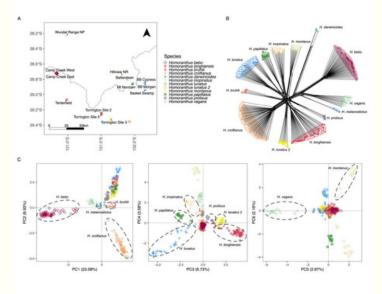
Isopogon fletcheri is a vulnerable species of shrub that has a disjunct distribution in the Blue Mountains of New South Wales, Australia. Previously known only from the cliffs of the Grose Valley, it has recently been discovered in a new population in the Kedumba Valley. These populations are separated by 17 km and occur in a region that is highly prone to bushfires. Other major threats include habitat loss and pathogens such as dieback caused by Phytophthora cinnamomi. Range disjunction, bushfires, and pathogens, can have significant genetic repercussions, directly impacting conservation planning. Genomics approaches present an unprecedented opportunity to explore genetic variation within species of conservation concern. As part of the Saving our Species (SoS) program, a conservation genomics study was conducted to examine genetic diversity, differentiation, and ancestry across the disjunct range of I. fletcheri, aiming to inform management recommendations. Our investigation revealed marked genomic differentiation between the Grose Valley subpopulations, and those in the Kedumba Valley. The observed limited gene flow, attributed to geographical disjunction and limited seed dispersal, suggests that the species has long persisted as isolated clusters. Across all subpopulations, evidence of inbreeding and diminished genomic diversity was evident, underscoring the urgent need for intervention. The compounded effects of isolation reduced genomic diversity, and inbreeding pose substantial threats to the species' long-term survival, necessitating proactive management strategies. Ex-situ germplasm collection has been identified as key action for the long-term persistence of Isopogon fletcheri. This study estimated genomically informed scenarios for selecting individuals that will maximize diversity in new ex-situ populations of various sizes.



Searching for Isopogon fletcheri along the cliffs of the Grose Valley, one of only two known poulations.

.....

We offer recommendations for managing these species, including suggestions for creating genetically diverse populations through translocation or ex-situ conservation. These steps are crucial for improving the survival chances of *H. bebo* and *H. croftianus* and for supporting their long-term conservation.



G Multispecies dataset analysis for Homoranthus.

Research Centre for Ecosystem Resilience Publications | 2024

### Alyssa Martini, Richard Dimon, Jia-Yee Samantha Yap, Maurizio Rossetto

Owenia cepiodora is a vulnerable tree species with a history of logging which has resulted in a fragmented distribution in the Border Ranges region of New South Wales and Queensland. This conservation genomic study, funded by the NSW government's Saving our Species (SoS) program, was conducted to update our understanding of the genetic diversity in the remaining populations of O. cepiodora, and investigate any loss in diversity in juvenile individuals. This study determined that Owenia cepiodora is characterised as having a single population that is genetically homogenous, and the species exhibits high relatedness of individuals within sites. Encouragingly, the level of genetic diversity determined in this study remains high, providing an opportunity to implement genetics-led conservation actions for this species to mitigate further reductions in overall fitness. To boost diversity for improving the overall health of the species, we recommend bringing together unrelated individuals, and have provided solutions to generate a genetically optimised collection that can be used as a source for translocation and augmentation at existing sites.

.....

## Conservation genomics of *Pomaderris cocoparrana* in support of management.

## Eilish McMaster, Caroline Cristofolini, Jia-Yee Samantha Yap, Maurizio Rossetto

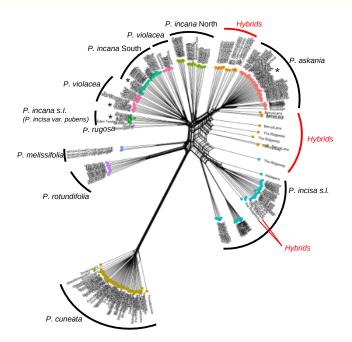
This report provides genomic insights into Pomaderris cocoparrana, an endangered shrub native to Cocoparra National Park, New South Wales, highlighting its genetic diversity, reproductive biology, and conservation strategies. The species, with fewer than 2,000 mature individuals as of 2014, is primarily found in Cocoparra National Park and faces threats from declining populations, herbivory, and poor fire management. Pomaderris cocoparrana shows significant genetic diversity and gene flow between sites, primarily due to outcrossing, which reduces inbreeding. However, this reliance on outcrossing makes the species susceptible to inbreeding depression if populations decline. Furthermore, our kinship analysis revealed no clones within the population, but some sites contained closely related individuals, which may reduce fertility over time if self-incompatibility mechanisms are present. Flow cytometry confirms that P. cocoparrana mainly reproduces sexually which enhances genetic diversity and adaptability, although some asexual seed production was observed. Given these findings, it is crucial to maintain a healthy wild population to prevent inbreeding depression. We also recommend establishing an ex-situ collection to safeguard the species' genetic diversity against threats like population decline, herbivory, and inappropriate fire regimes. This collection can be used for future translocations or site augmentation as well. Proposed sampling aims to capture over 95% (n=13) and 99% (n=23) of common allele diversity, ensuring the long-term viability of P. cocoparrana.

Conservation genomics of *Prostanthera askania* in support of management.

### Caroline Cristofolini, Jia-Yee Samantha Yap, Trevor Wilson, Maurizio Rossetto

The endangered Prostanthera askania exhibits a remarkably limited distribution in the Central Coast region in NSW, with most populations occurring outside conservation areas where it is threatened by urban and industrial development. The Research Centre for Ecosystem Resilience (ReCER) conducted a conservation genomics study to obtain insights into the species status and genetic health of Prostanthera askania. The study confirmed P. askania as a genetically distinct species using a comprehensive sampling approach that clarified its distinction from a broad range of morphologically variable representatives of P. incana s.l., P. violacea and P. incisa s.l. The distribution of genetic diversity in P. askania indicates the species can be managed as two populations, and we advocate for management strategies that prioritise and enhance population connectivity. Considering the unique genetic makeup of Bouddi National Park population, genetic rescue efforts should be explored. Hybridisation poses a significant threat to the long-term viability of P. askania if left unmanaged. The genomic analyses showed that some sites consisted of only hybrids and these hybrids more likely to have resulted from mating between P. askania and P. incana despite the co-occurrence of P. askania with P. incisa. Relying solely on morphology as a metric for detecting hybrids will not always yield reliable results. As a result, it is imperative that any cultivated P. incana in proximity to P. askania be removed to mitigate the risk of genetic swamping and preserve the integrity of remaining P. askania

populations. If ex-situ collections were to be generated, we highly recommend conducting genetic screening. It is crucial to tag the individuals during the sampling phase of a genetic study and identify the genetically pure individuals for the collection. Knowing where the genetically pure individuals reside in the wild, allows them to be revisited to collect material for the collection when needed and facilitates more robust long-term monitoring of population dynamics.



• Genomic research detected hybridisation that poses a significant threat to the long-term viability of *Prosanthera askania* if left unmanaged.

## Conservation genomics of *Zieria baeuerlenii* in support of ex-situ management.

### Jia-Yee Samantha Yap, Maurizio Rossetto

Zieria baeuerlenii is an endangered shrub confined to an extremely small population along both sides of the Bomaderry Creek in New South Wales. This species has not been observed to produce seed, making its conservation reliant on maintaining genetic diversity ex-situ across various Botanic Gardens. This conservation genomic study, funded by the NSW government's Saving our Species (SoS) program, was conducted to update our understanding of its in-situ genetic diversity and to refine the management of its ex-situ collection. The study corroborates previous phylogenetic studies by confirming that the species is genetically distinct. However, it also reveals that the species is even rarer than previously understood, with only 18 genets remaining. This new information has significant cost-benefit implications for existing ex-situ collections, in that a reduction in the number of ramets held ex-situ should be implemented for each genet and also there is value in keeping the plants that this study demonstrate are representatives of distinct genets. We propose that while re-evaluating the representation of each genet in the collection that any excess plants and aging stock be used as new plantings (i.e., enhancement planting site or in situ population). We recommend also to ensure that all genets are represented in these new plantings and implement a robust tagging system of individual plants to track the surviving genets and facilitate long-term monitoring. Although not within the scope of this study, we recommend prioritising an assessment of pollen viability across all Z. baeuerlenii genets to explore possibilities to reinitiate sexual reproduction, which is essential for the species' long term viability



Genomic research revealed Zieria baeuerlenii is rarer previously understood and is helping to ensure ex-situ collections are representative and avoid unecessary duplication of genets.

### Other unpublished reports

### Resilient trees, resilient woodlands: Box Gum Woodland Tree Knowledge Infrastructure.

#### Patrick Fahey, Patricia Hogbin, Marlien van der Merwe, Maurizio Rossetto

This report provides the findings and guidelines arising from the Resilient trees, resilient woodlands: Box Gum Woodland Tree Knowledge Infrastructure project, a collaboration between Research Centre of Ecosystem Resilience (ReCER), Botanic Gardens of Sydney and Taronga Conservation Society Australia. Restoration of koala habitat is a key tool in ensuring the long-term survival of koalas in the wild, especially in the face of a rapidly changing climate. Genetic information can help ensure material used in habitat restoration is of appropriate provenance and sufficiently genetically diverse. Without genetic guidance, practitioners are limited to unreliable generalisations hat may underestimate appropriate seed collection areas and may lead to genetically depauperate restored populations with reduced fitness & low adaptability. These guidelines provide advice on how to source genetically diverse climate-ready seed for Eucalyptus species that are associated with the critically endangered Box-Gum Grassy Woodland ecological community and will help ensure the long-term self-sustainability of Koala habitat restoration being undertaken by the Taronga Conservation Society Australia.





### Snapshot of methodology.

Eleven dominant and widespread woodland eucalypt species, suitable for use in restoration of Box-Gum Grassy Woodland in the Northern Tablelands of New South Wales, were sampled from across their distribution in New South Wales. 1812 leaf samples were collected from across 332 sites. DNA extraction and DArT sequencing was undertaken for these and outgroup samples. Taxonomic and distributional boundaries were tested, and population genetic analyses undertaken. Future climate matched locations were identified and combined with genetic data to determine an optimised seed sourcing strategy for each of the 11 target eucalypts that captures high levels of genetic diversity and incorporates severe climate readiness.

The genetic analyses proved a powerful tool for highlighting and resolving taxonomic and distributional uncertainty for several target woodland species, revealing that not all target taxa may be suitable for planting at the target restoration site and that careful attention need to be paid to refined species boundaries and distributions when planning seed collection for use across the Northern Tablelands.

Poulation genetic analyses revealed that for all species investigated, gene flow has historically been largely continuous across the tableland and inland slope regions of NSW. While consistent evolutionary patterns are seen across the target species, this is the result of this study being limited to ecologically dominant eucalypts, which share similar patterns of gene flow and genetic diversity levels across species and cannot be generalised to other taxonomic groups which occur in the Box-Gum Grassy Woodlands. Only the Hunter Valley proved to be a major barrier to geneflow for four of the eleven species, and therefore the resultant evolutionary divergence needs to be considered when moving seed across this region for use in restoration. In line with previous restoration genomics studies in eastern Australian woodland plants, there was no signal of local adaptation detected using the neutral genetic markers, with geographic distance being the key driver of genetic patterns. Therefore, targeted mixing of seed from multiple sources from both local to a restoration site and from the wider region (including climate ready material) is recommended for all species. This strategy will minimise any negative genetic consequences and will increase the accessibility and genetic diversity of seed. Furthermore, while not something we tested directly in this project, it has been established that a relationship exists between source population size and genetic diversity, meaning that seed should be sourced from the largest, most intact populations possible.

Ten key steps for sourcing genetically diverse climate-ready seed for use in restoration of Box-Gum Grassy Woodland are discussed, including that the number of mother trees seed are collected from (maternal lines) be maximised and that seed from each maternal line be tracked and kept separate throughout the entire process of seed collection to restoration planting. It is also important to avoid sourcing seed from small, planted or hybridising populations. The ideal spatial distribution of seed source sites and mother plant are outlined, including that seed source sites be separated by at least 20 km and mother trees within sites should be at least 100 m apart. A planting block design that maximises the chance of establishing genetically healthy and self-sustaining restored populations is presented. Detailed findings regarding these species' evolutionary genetics and reproductive biology, and species-specific guidelines for optimised seed collections are provided for all 11 target species. Eight species for which we present specific guidelines here have been added to the publicly available Restore and Renew webtool, allowing restoration practitioners to identify appropriate seed sourcing regions for specific restoration projects not only within Box-Gum Grassy Woodlands, but all vegetation communities in which the target species occur. The genetic datasets for three remaining species were not appropriate for the model employed by the webtool and so they could not be deployed there at this time (but will be in an upgrade of the webtool that is currently being developed).

The insight gained from this research can benefit not only the restoration of eucalypts for Box-Gum Grassy Woodland on the Northern Tablelands, but eucalypt restoration in general, thereby improving the effectiveness of on-ground Koala conservation action across NSW and beyond. We finish by highlighting the value of raising awareness regarding how genomic research can improve restoration of eucalypts and identify two key areas for future research (i) develop seed sourcing guidelines for other Box-Gum Grassy Woodland species, and (ii) evaluate and monitor feasibility and effectiveness of recommendations.

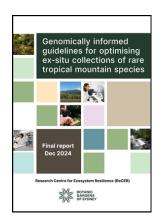


Summary of ten key steps for genetically diverse climate-ready restoration of Box-Gum Grassy Woodland. Research Centre for Ecosystem Resilience Publications | 2024

## Genomically informed guidelines for optimising ex-situ collections of rare mountain species.

### Manuela Cascini, Maurizio Rossetto

This document provides genomic data and ex-situ management guidelines for ten tropical mountain species analysed as part of the Tropical Mountain Plant Science Project (TroMPS, <u>https://www.tromps.org.au</u>). The species included are: *Cinamomum propinquum*, *Cryptcarya bellendenkaran*, *Dracophyllum sayeri*, *Eucryphia wilkiei*, *Flindersia oppositifolia*, *Litsea granitica*, *Polyscias bellendenkerensis*, *Rhodamnia longisepala*, *Uromyrtus metrosideros*, and *Zieria alata*. The study was conducted by the Research Centre for Ecosystem Resilience (ReCER) at the Botanic Gardens of Sydney in collaboration with the Australian Tropical Herbarium (James Cook University, Cairns, Queensland). Population genomics methods developed by the ReCER team were used to assess the representativeness of existing ex-situ collections and to inform the design of genetically optimised collections to improve both existing and future ex-situ collections and potential translocations.



## Conservation genomics of *Pultenaea imminuta* in support of management.

### Saphira Bloom-Quinn, Marlien van der Merwe

Pultenaea imminuta (R. L. Barrett & S. F. McCune) is a recently described species that emerged from the taxonomic revision of the Pultenaea setulosa species complex (Barrett et al. 2024). The shrub has an extremely restricted range, found at only three sites in Leard State Forest in north-western New South Wales. The sites are near each other and to Boggabri Coal Mine and Maules Creek Coal Mine and were identified during pre-clearing flora surveys in February 2024. Two of the sites contained enough individuals to maintain a viable population while only a small number of individuals persisted at the third site. Fire, logging and weeds have been reported as threats (Barrett et al. 2024). Based on these factors, Pultenaea imminuta is likely to satisfy the IUCN criteria for a Critically Endangered species (Barrett et al. 2024) and is being nominated for listing. This conservation genomics study aims to provide genetic information about the two main confirmed Pultenaea imminuta sites: Leard State Forest and Biodiversity Corridor, which were sampled at the time of the Leard State Forest site clearing in 2024. Based on the genetic data, advice is provided for the use of ex-situ nursery material held by Fields Environmental Solutions from the Leard State Forest, and establishment of a translocation site.

Analysis of the data revealed that while the sites are genetically distinguishable, the level of differentiation between sites was low. Material from the two sites can be mixed for translocations. While moderately high levels of genetic diversity were detected at each site, inbreeding was also indicated. Based on a kinship analysis, no related individuals were detected. Therefore, management of in-situ and ex-situ plants should take care to avoid inbreeding. Avoid planting cuttings from the same plant at the same sites or in close proximity. Estimates indicate that each site contained 3000+ private genetic variants (alleles) that are not found in the other site. Leard State Forest holds unique genetic diversity not present at the Biodiversity Corridor. This diversity represents at least 10% of the total genetic diversity of the species. We provide guidelines on ratios of plants from the two sites to be used when creating new populations and recommend that genetically optimised collections should predominantly include individuals from the Biodiversity Corridor with a recommended proportion of 30% unique Leard State Forest individuals and 70% unique Biodiversity Corridor individuals. We recommend the establishment of multiple new populations each containing a mix of unrelated individuals.

### **Blog posts**

## Genomics enhancing native plant restoration and conservation.

### Tricia Hogbin

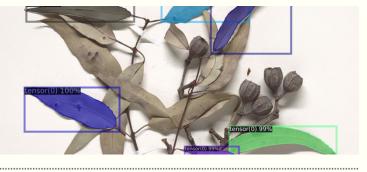
Five years ago, ReCER published a seminal paper describing how to gather and interpret vast amounts of genomic data in support of ecological restoration. Since then, leaf samples have been collected from 89,000 plants and a growing database is supporting the restoration and conservation of more than 130 Australian plant species. The ability to collect and sequence the DNA of tens of thousands of plant samples is not the only ground-breaking aspect of the Restore and Renew Knowledge Infrastructure project; what makes this ambitious project most unique is that it is the first tool globally to make genomic guidance freely available for restoration practitioners, community groups and others who are restoring native vegetation. <u>Read more</u>



#### .....

### <u>Al revolutionising scientists' understanding of nature.</u>

New developments in machine learning are allowing a generation of vast datasets to reveal changes in plants. Using machine learning, researchers are creating new methodologies to analyse datasets of unprecedented size. A team from Botanic Gardens of Sydney reveal how their latest study, focused on the leaves of eucalyptus trees, details how an iconic native species has evolved with climate. Read more



### Rainforest Connections conference highlights.

Among delegates of the recent Rainforest Connections 2024 conference were four researchers from ReCER. They shared their conference highlights and also a summary of their presentations, illustrating the diverse ways genomic research by ReCER is contributing to rainforest conservation and restoration. For example, Maurizio Rossetto spoke about the implications of evolutionary history for Gondwanan rainforest conservation, Richard Dimon outlined how genomic workflows are informing the sourcing of material for restoration, Manuela Cascini presented several case studies of how genomics is guiding the conservation planning and management of threatened rainforest plants, and Monica Fahey delivered a presentation on restoring the adaptive potential and cultural connectivity of edible rainforest trees. <u>Read more</u>



#### Eucalypt restoration improved by genomic guidelines.

#### **Tricia Hogbin**

Over the past five years, leaf samples have been collected for genetic analysis from almost 10,000 eucalypt trees across New South Wales. A unique barcode keeps track of each leaf sample as it goes through a process of DNA extraction, sequencing, and analysis. The resulting genetic data is combined with climatic data within the Restore and Renew webtool, which can be used by restoration practitioners to consider genetic information and climate change when sourcing seed and other material for restoration. Currently, 19 eucalypt species (with more to come) are available for seed sourcing guidance on the webtool. Read more



### <u>Restore and Renew webtool showcased to international</u> <u>audience.</u>

The Restore and Renew webtool, designed to help practitioners apply genetic information and consider climate change when sourcing material for restoration, was among 13 case studies showcased in international guidelines for tree genetic resource conservation. Achieving the restoration needed to address the triple crisis of failing food systems, biodiversity loss, and climate change requires the establishment of billions of trees on millions of hectares of degraded land, suggests the working paper. "But what we plant matters" explains ReCER Head Maurizio Rossetto. In the working paper, Maurizio shares the Restore and Renew workflow process, highlighting how one dataset can answer multiple questions for multiple species, increasing economy of scale, and making genetic information more accessible. <u>Read more</u>



## New genomic research highlights Indigenous ecological knowledge.

#### **Monica Fahey**

A collaborative cross-cultural research team have discovered that movement of the culturally significant Bunya Pine by Indigenous Peoples varied among regions and intensified in south-east Queensland to maintain cultural connectivity following European colonisation. Translocation, or the movement of plant propagules from one place to another, is becoming an increasingly important tool in conservation, especially given the need to help species adapt to a changing climate."Our findings contribute to a broader understanding of the demographic and genetic impacts of translocations and can be combined with Indigenous knowledge to design a conservation strategy for Bunya Pine that incorporates both biological and cultural information" says ReCER Research fellow Monica Fahey. <u>Read more</u>



### Selected media features

### Science Saving rainforests project in action.

Genomic research by ReCER is guiding selection of 'mother trees' for the Science Saving rainforests project by the Big Scrub Rainforest Conservancy. Leaves have been collected for genetic analysis from more than 10,000 trees from 60 plant species from Far North Queensland to southern New South Wales. <u>Watch</u>



### Rescuing our endangered plants, Ku-ring-gai Council.

In this video, Ku-ring-gai Council staff share how they are working to protect and propagate the threatened plant 'Hal' *Haloragodendron lucassii*. Caroline Cristofolini shares show genetic information is helping guide recovery efforts. <u>Watch</u>



## Subscribe

### **ReCER** e-newsletter

Research updates for restoration and conservation practitioners and policymakers

Sign up to the occasional ReCER e-newsletter for practitioner and policymaker updates, new publications, news, events and opportunities.

Subscribe here: https://www.botanicgardens.org.au/recer

