Threatened Species Recovery Hub

National Environmental Science Programme

Genetic assessment of bushfire-impacted vertebrate species

Final Report

Renee Catullo and Craig Moritz

July 2021

THE UNIVERSITY OF WESTERN AUSTRALIA

Australian National University

Cite this publication as: Catullo, R., Moritz, C., 2021. Genetic assessment of bushfire-impacted vertebrate species. NESP Threatened Species Recovery Hub Project 8.3.3 Final report, Brisbane.

Cover image: Broad-headed snake (*Hoplocephalus bungaroides*). Image: Alan, CC BY 2.0 Wikimedia Commons

Contents

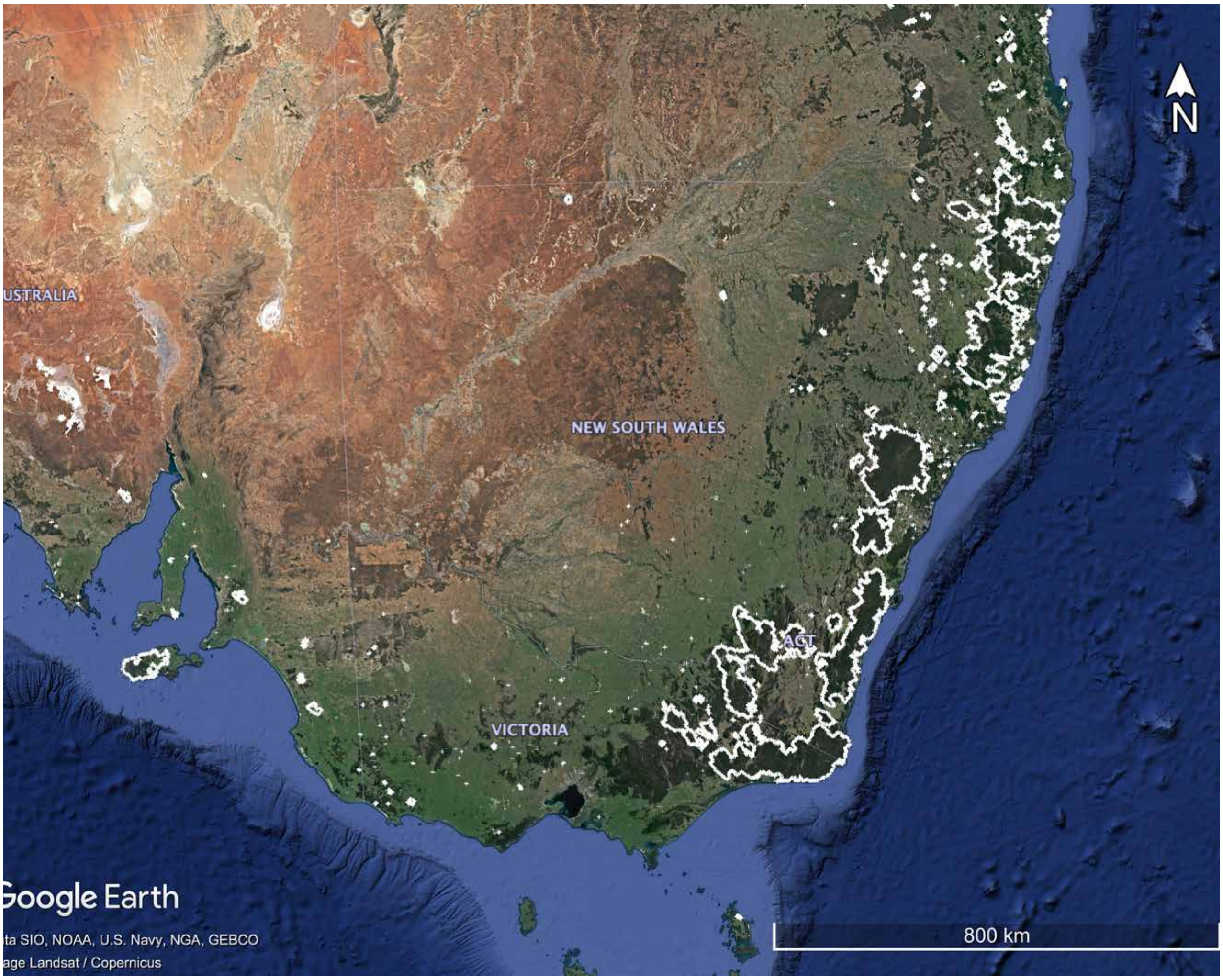
Executive Summary

The 2019-2020 bushfires had severe impacts on many animal species, with many experiencing substantial reductions in their population size and habitat. As a result, the federal government led a rapid assessment of the impacts on vertebrate species, prioritising emergency action for those whose distributions were substantially fire-affected. However, genetic assessments of these species identified that the taxonomic definitions used were in some cases incorrect, leading to mis-prioritisation. Unrecognised cryptic diversity can lead to the unintentional loss of unknown species following catastrophic events.

This project sought to provide genetic information to improve the understanding of diversity across bushfire-affected vertebrates. We targeted species identified as priorities by the federal government and experts, collating existing genetic data to identify structure or endemism relevant to the prioritisation and management of fire-affected species. This enabled us to assess the number and distribution of conservation units within species, or of as-yet under-described species, sampling gaps in which taxa or units present is unknown, and provide advice on which areas and species are likely to be most impacted by the bushfires. We also provided spatially explicit advice on where additional sampling is needed to better assess conservation genetic requirements.

Following this, we worked with museums and universities to generate landscape genetic information for approximately 50 priority species. This component, still in progress, will provide greater understanding of the taxonomy and genetic structure of these species. We also ran a workshop with federal and state conservation agencies to understand how best to use these data to improve conservation advices and management. These insights will be incorporated into our future species assessments using the new landscape genetic information.

Our project has provided information to assist with the reassessment of the current threat listings of bushfire-affected species. The information, including detailed maps and summaries for each species, has also been provided to state agencies responsible for the conservation management of bushfire-affected species.



A map of the study location.

Introduction

The 2019-2020 bushfires had severe impacts on many animal species. Some of these species were considered threatened prior to the fires, and as a consequence of the fires may have lost a substantial proportion of their remaining population and habitat. For other species, not previously considered threatened, substantial reductions in population size and habitat may lead to their reassessment as threatened. As a result of the fires, the Department of Agriculture, Water and the Environment (DAWE) rapidly developed a draft framework to evaluate and prioritise emergency action for all vertebrate species whose distributions were substantially fire-affected (Legge et al., 2020). The published draft framework ranks species for priority action using a combination of data: the overlap of the species with fire, pre-fire threat status, traits that influence during- and post-fire mortality, and the likelihood of species' recovery. This detailed prioritisation was carried out on threatened or migratory taxa with more than 10% of their known and likely distribution in fire-affected areas, and unlisted taxa where more than 30% of the distribution was fire affected. Both species and subspecies were assessed for birds.

The draft framework provided an important and consistent method for prioritising taxa for management interventions. However, genetic assessments of vertebrate species conducted by an expert working group assembled by the Centre for Biodiversity Analysis in April 2021, identified that the species and subspecies definitions on which the initial fire-affected distributions were calculated were in some cases misleading. These genetic data revealed a number of issues that could have led to mis-prioritisation of species, or to highly impacted species not being assessed.
Genetic assessments of fire-affected species have revealed that:

- Some species are incorrectly defined using traditional (mostly morphological) taxonomy.
    - A significant number of "species", particularly in morphologically uniform groups such as frogs and reptiles, are species complexes with one or more currently undescribed species.
    - Subspecies often do not form unique genetic clades and can have less genetic divergence than geographic populations within subspecies.
- Species are not genetically uniform across geography. Well-defined species have multiple independently evolving segments within their geographic ranges that are not typically represented by taxonomy (e.g. not defined as subspecies).
- The use of subspecies as a taxonomic category is not consistent across taxonomic groups. In particular, subspecies are more commonly described formally in birds than in other taxonomic groups.

Correct identification of both taxonomic and genetic diversity has significant implications for the long-term recovery and persistence of threatened species (Coates, Byrne and Moritz, 2018). Unrecognised 'cryptic' diversity can lead to the unintentional loss of unknown species following catastrophic events. The maintenance of high levels of genetic diversity in and between populations helps species recover and avoid inbreeding depression. These issues are especially prevalent in low-dispersal species along the east coast of Australia where there are current and historical barriers to dispersal that contribute to the evolution of diversity between isolated populations (Chapple et al., 2011). Consideration of this diversity is essential if we are to avoid cryptic extinction and so lose large components of the genetic diversity within species.

Over-splitting of taxonomic units (as species or subspecies) can also have detrimental effects for long term persistence through deterring managed admixture between threatened and declining populations. Declining populations are subject to the combined effects of inbreeding depression and genetic drift, which reduce the probability of survival. Management efforts such as genetic rescue endeavour to increase background genetic diversity within populations while maintaining adaptive differences through targeted translocations (Kriesner et al., 2020). Genetic rescue has been shown to increase the genetic diversity and abundance of threatened populations through increased offspring fitness, helping reduce the risk of extinction (Weeks et al., 2017).

Understanding genetic diversity across species and populations therefore allows a more complete prioritisation and management of threatened species. It prevents the accidental loss of currently undescribed species or "important populations" within species by recognising highly distinct genetic units (Moritz, 1994). Acknowledging and understanding patterns of genetic diversity can also allow managers to make calculated decisions about admixture between specified taxonomic units in order to genetically rescue declining populations (Moritz, 1999).

For many species, the ability to assess patterns of genetic diversity is limited by poor sampling of tissues. Most genetic studies rely on tissues stored in our history collections. However, the amount and geographic distribution of samples for many species is relatively low or patchily distributed relative to known occurrences. These gaps in sampling can mislead assessments of conservation units or otherwise prevent the inclusion of valuable information about genetic diversity in conservation assessment.

To address these challenges, this project worked to provide information to improve understanding of diversity across bushfire-affected vertebrates. This included providing species-level assessment of species with pre-existing relevant genetic data, identifying species where genetic data is likely unhelpful to planning, identifying areas where additional sampling is necessary, and prioritizing species for generating additional landscape genetic data subject to availability of samples.

Methodology

This project had two major stages, which enabled the rapid incorporation of information on taxonomic and genetic diversity into listing assessments and rapid post-bushfire conservation management. In the first stage, we rapidly reported on genetic diversity of priority species using pre-existing genetic data. In the second stage of the project, we identified priority species given high priority for further genetic assessment in the initial workshop and which had sufficient existing tissues for more in-depth landscape genomic analyses. For these taxa we obtained samples and organised additional genomic data to be generated. In parallel, we developed pipelines to convert the genomic data to useful information and visualisations. For a subset of priority species, we then ran a workshop with federal and state conservation managers to assess the usefulness of the statistics and visualisation and how to use these data to inform future conservation actions through conservation advices.

Genetic diversity is hugely important in recovery and long-term persistence of bushfire-affected species, and the initial assessment provided that information to relevant state and government agencies for 59 species (Table 1; https://www.nespthreatenedspecies.edu.au/projects/genetic-assessment-of-priority-taxa-and-management-priorities). This assessment includes information on species where genetic data, often so far unpublished, provides information that is relevant to prioritisation and management. These data included:

- Information on highly genetically divergent lineages that likely represent undescribed species;
- Identification of subspecies with little genetic support, where separate management should be reassessed;
- Detailed data on genetic diversity and genetic structure that is relevant to conservation management.

Table 1. Species ranked by experts as a high priority for additional sequencing, or medium/high for Kangaroo Island. Species with some existing data and therefore included in initial assessments are marked with an asterisk. Kangaroo Island priorities are marked by a +.

	Genus	Species
Frogs	*Crinia*	*signifera**
	Litoria	*olongburensis**
	Mixophyes	*iteratus*
	Pseudophryne	*pengilleyi*
	Adelotus	*brevis*
	Crinia	*tinnula**
	Limnodynastes	*dumerilii*+*
	Litoria	*ewingii*+*
	Philoria	*kundagungan**
	Philoria	*loveridgei**
	Philoria	*pughi**
	Philoria	*richmondensis**
	Philoria	*sphagnicola**
	Litoria	*piperata*
	Pseudophryne	*bibroni+*
	Pseudophryne	*corroboree*
	Pseudophryne	*dendyi*
Reptiles	*Anepischetosia*	*maccoyi*
	Eulamprus	*heatwolei**
	Eulamprus	*kosciuskoi**
	Eulamprus	*leuraensis**
	Eulamprus	*tympanum**
	Pseudemoia	*cryodroma**
	Pseudemoia	*pagenstecheri A*
	Saiphos	*equalis*
	Saltuarius	*kateae*
	Saltuarius	*moritzii**
	Saltuarius	*wyberba*
	Cyclodomorphus	*michaeli*
	Coeranoscincus	*reticulatus*
	Harrisoniascincus	*zia**
	Intellagama	*leseurii*
	Liopholis	*whitii*
	Phyllurus	*caudiannulatus*
	Phyllurus	*platurus*
	Pseudemoia	*rawlinsoni*
	Varanus	*rosenbergi*+*

	Genus	Species
Mammals	*Antechinus*	*argentus**
	Antechinus	*arktos**
	Cercartetus	*nanus*+*
	Cercartetus	*lepidus*+*
	Dasyurus	*maculatus*
	Petaurus	*breviceps**
	Petrogale	*penicillata**
	Pseudomys	*fumeus**
	Antechinus	*mimetes**
	Mastacomys	*fuscus*
	Potorous	*longipes*
	Potorous	*tridactylus**
	Rattus	*lutreolus+*
	Sminthopsis	*fulignosus aitkeni+*
	Vespadelus	*darlingtoni+*
	Vespadelus	*regulus+*
Birds	*Anthochaera*	*chrysoptera +*
	Calyptorhynchus	*lathami* +*
	Hylacola	*cauta halmaturina+*
	Psophodes	*nigrogularis lashmari*+*
	Pezoporus	*wallicus**
	Stipiturus	*malachurus*+*
	Zoothera	*lunulata halmaturina+*

Following the initial report, we identified priority species with sufficient existing samples for further detailed genomic analysis. For these species, we worked with museum curators and university researchers to bring together tissue samples and existing genomic data for ~50 priority species (Table 2). This required a complex combination of reanalysing existing genetic data, extending previous datasets with new sequencing and bioinformatics, and undertaking entirely new genetic analyses for species with no existing genetic datasets.

Given COVID-related lockdown issues affecting access to key museum collections, we experienced substantial delays in acquiring the necessary tissues. The tissues were sent by all collaborating institutions to the Australian National University, where we undertook DNA extractions for all species. These extractions were sent to Diversity Arrays Pty Ltd and the Australian Genome Research Facility for sequencing. Draft genomic data was returned in July 2021, with final genomic data expected in September 2021. In preparation for the return of these data, we consulted with conservation managers on how to best use landscape genetic information.

Table 2. Species included in the second round of analyses. These are priority species with sufficient existing samples for further detailed genomic analysis.

	Scientific Name	Common Name
Mammals	*Antechinus stuartii*	Brown antechinus
	Antechinus subtropicus	Subtropical antechinus
	Antechinus agilis	Agile antechinus
	Antechinus argentus	Silver-headed antechinus
	Dasyurus maculatus	Spotted-tailed quoll
	Cercartetus nanus	Eastern Pygmy Possum
	Petauroides volans	Greater glider
	Petrogale penicillata	Brush-tailed rock-wallaby
	Potorous longipes	Long-footed potoroo
	Potorus tridactylus	Long-nosed potoroo
Birds	*Menura novaehollandiae*	Superb lyrebird
	Menura alberti	Albert's lyrebird
Reptiles	*Hoplocephalus bungaroides*	Broad-headed snake
	Egernia saxatilis	Black rock skink
	Egernia roomi	Kaputar rock skink
	Anepischetosia maccoyi	Highlands forest-skink
	Harrisoniascincus zia	Rainforest cool-skink
Frogs	*Assa darlingtonia*	Marsupial frog
	Adelotus brevis	Tusked frog
	Crinia signifera	Eastern common froglet
	Crinia tinnula	Wallum froglet
	Limnodynastes dumerilli	Banjo frog
	Litoria booroolongensis	Booroolong frog
	Litoria dentata	Bleating tree frog
	Litoria ewingii	Ewing's tree frog
	Litoria littlejohni	Heath tree frog
	Litoria olongburensis	Wallum sedge frog
	Litoria verreauxii	Whistling tree frog
	Litoria watsoni	Watson's tree frog
	Mixophyes iteratus	Giant barred frog
	Philoria kundagungan	Red and yellow mountain frog
	Philoria loveridgei	Masked mountain frog
	Philoria pughi	Pugh's mountain frog
	Philoria richmondensis	Richmond range mountain frog
	Philoria sphagnicolous	Sphagnum frog
	Heleioporus australiacus	Giant burrowing frog

In June 2021, we held an online workshop to explore the ways genetic information can support the work of conservation managers. The workshop was held over two days and included 36 participants, over half from conservation management organisations including the Australian Wildlife Conservancy, Department of Agriculture, Water and the Environment, and all state environment departments. The aim of the workshop was to identify the key questions we should answer with the genetic data collected for the high-priority species, to test the value of our approach to visualising and communicating genetic diversity information to managers, and how to incorporate the answers into government policies and statutory documents, using three exemplar species as examples.

Outputs Findings

Interim report

The first output was an initial report on the forthcoming changes in species distributions, within species genetic structure, and recommendations for further field sampling to help assess genetic risk. In this report, we provide maps of priority taxa and their major genetic populations, including areas of uncertainty where further sampling is necessary.

The interim report (published September 2020) used the initial expert evaluation of 202 species from the priority assessment list identified by state and federal agencies (Legge et al., 2020) and experts to provide genetic information relevant to conservation management. The report identifies 32 species known (from genetic evidence) or very likely (based on species' biology) to be a single species that lacks substantial genetic structure across the fire-affected areas. These species should be assessed for fire impacts as a single entity.

The interim report then identifies 59 high-priority species for which existing genetic data provides relevant information for conservation management and monitoring priorities in bushfire-affected areas. The interim report provides, in a confidential appendix, detailed assessments of genetic structure and mapping of conservation units for these 59 species, and information on high-value genetic populations where this is available. These are species for which existing genetic data provides relevant information for conservation management and monitoring priorities in bushfire-affected areas.

Across these 59 species, as well as many of the other high-priority species on the original priority assessment list, genetic sampling is not available for all areas of the species' distribution. The interim report categorises these areas where genetic data is missing, both by summarising the areas with the poorest sampling across taxa, and also providing lists, by protected area, of species that require sampling.

Workshop

Project outputs two and three related to running a workshop with managers (held June 2021), and reporting on workshop outcomes (this report). This workshop was initially focussed on reviewing new evidence on species/ESU limits, mapping of endemism hotspots, and assessing genetic risk following fire impacts. However, following consultation with conservation managers, the workshop aim was altered to focus on how to provide genetic information relevant to on-ground conservation actions. This is where the managers saw the greatest value coming from our work.

The workshop focussed on the types of genetic analyses and interpretations needed for policy-making, with the discussion focussed on how the variation in threat status, major threats, and the ecology of the species influenced the information required. The key emergent theme from the workshop was communication - when to communicate, who to communicate with, and what to communicate. Conservation managers saw significant value in the types of information geneticists can provide for species management, particularly if communication was done appropriately.

The first key outcome is that conservation managers need to be involved in all levels of genetic research, particularly the design of studies and communication of results. Involving conservation managers in the design of genetic studies currently faces a number of barriers. The first challenge, faced by both sides, is understanding who to speak to. Conservation managers identified that it was impossible to know what relevant research was occurring, and who to contact for taxa of interest. Geneticists faced similar barriers, finding it difficult to identify relevant individuals within government agencies due to a lack of conservation manager visibility within departments, as well as staff turnover in government. One key idea identified was the generation of a register of research, perhaps hosted via museums or the Atlas of Living Australia, to enable researchers and conservation managers to find each other.

Prior discussion of sampling design was identified as highly important by conservation managers. These conversations would enable conservation managers to provide feedback on the outcomes of the analyses, such including past and present sampling (e.g., for assessing translocations) and information needed to assess genetic health (e.g., heterozygosity or private alleles).

A recurring theme of the workshop was the lack of accessibility of genetic data and analyses at times when it was urgently required. This was considered a challenge created by the time-sensitive nature of conservation actions, versus the time-consuming nature of research and publication. While researchers were in general willing to share unpublished information with conservation managers prior to publication, this process requires better development of methods to identify key contributors from both groups.

Several challenges were identified when genetic information is being provided for use in conservation. First, conservation managers often lack the time, resources or expertise to interpret genetic results. To ensure that research is able to be easily used in policy, participants discussed the need for conservation inferences to be clearly and succinctly stated when reporting scientific results. It is important that both the robustness and uncertainty within data are explained in clear terms. For example, stating whether genetic sampling is representative of the entire species distribution, or distinguishing between past and present sampling, would assist conservation managers in assessing the usefulness of the data. Participants raised the need for researchers to refer to policy documents such as the IUCN Listing Criteria when designing and communicating their research, to ensure that the research provides useful results to managers.

Participants discussed the importance of being mindful of the language used when reporting genetic results. Language needs to be clear, with limited jargon and technical terms defined to provide important clarity around actions. Many terms may have different meanings for geneticists, managers and policy-makers, which may lead to misunderstanding and to unintended downstream consequences. For example, the term ‘population’ has a specific meaning under legislation, which may not align with the genetic understanding of the term. Using this term may have the unintended consequence of implying that individuals from these distinct groups cannot be mixed, preventing genetic rescue, although this may not align with the geneticist’s view.

The “completeness” of the advice provided by geneticists is considered extremely important to avoiding unintended consequences. These consequences are generated due to interpretations being equiivalent of “because it wasn’t stated, don’t do it”. For example, if a particular population is recommended as a source for translocations to another population, it is important to address whether any other populations are also suitable but not preferred sources, or whether they should not be used. Without addressing this specifically, the presumption is generally that the other populations should not be used, and this may unintentionally limit conservation actions that would generally be suitable, if not optimal. Therefore it is important to provide complete advice that ranks actions to ensure all suitable management actions remain accessible if needed.

The workshop focussed on assessing genetic risk across three example threatened species with differing biology. These species represented a previously widespread but now fragmented species (New Holland Mouse), a short-range endemic (Littlejohn’s Tree Frog), and a naturally fragmented species (Marsupial Frog). The genetic advice developed in collaboration with geneticists and conservation managers for the Marsupial frog *Assa darlingtoni* is included in Box 1 as an example. This advice incorporates the key recommendations from the workshop for genetic assessment of a species, and will represent a framework to be applied to the other species undergoing sequencing (Table 2).

Future work

Several key outcomes are ongoing from this project, as they require the return of the newly generated landscape genomic data and then processing of these data though the analysis and visualisation pipeline (see Fig. 1). First is the generation of integrated maps of endemism hotspots, including weighting of taxa by their evolutionary divergence. Secondly for selected taxa, results of the genetic risk assessment as defined by Kriesner et al. will be carried out using the newly generated data. In the interim, as new results become available for impacted species, these will be communicated immediately to our network of agency staff who are continuing to prepare conservation advices.

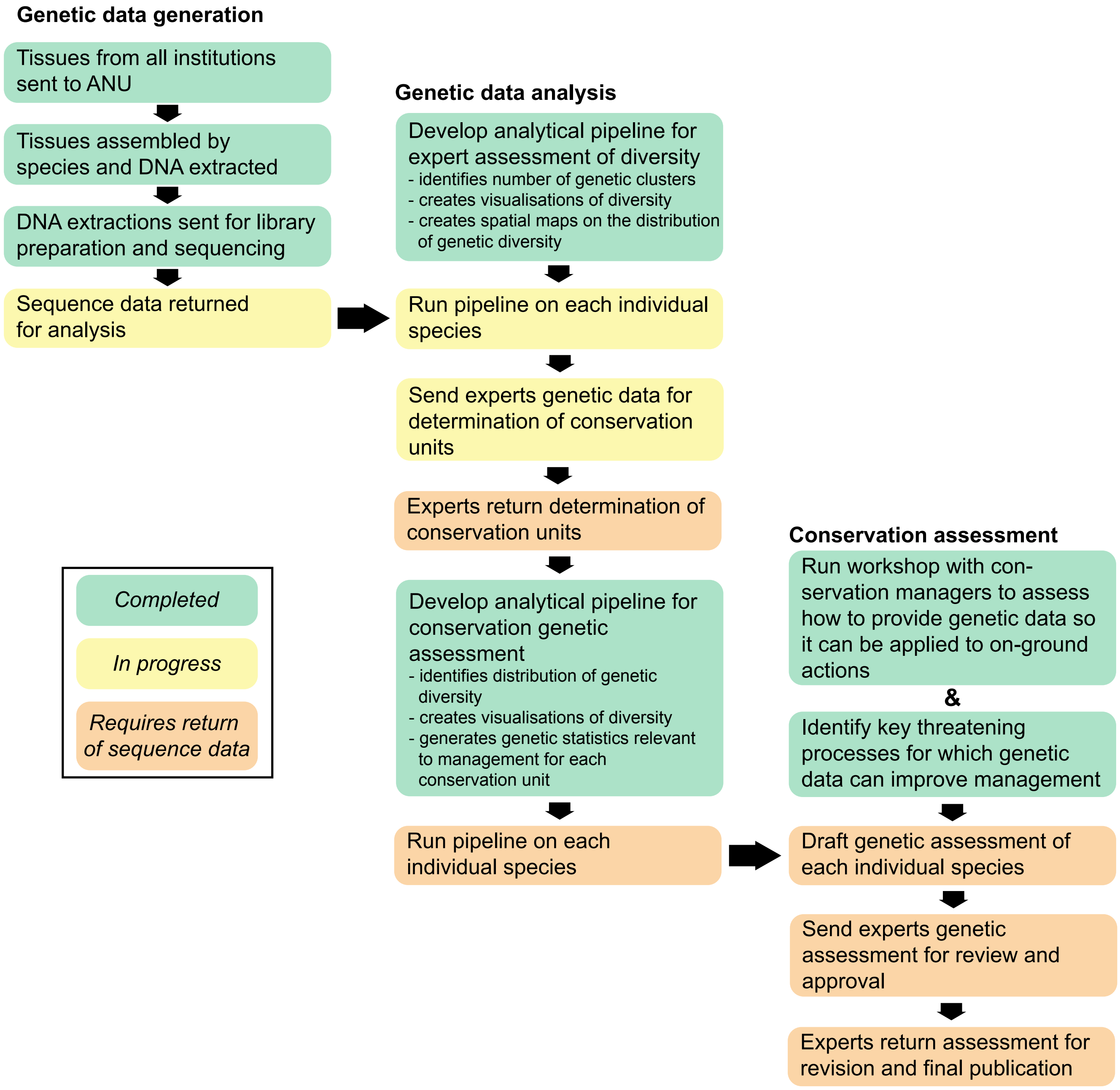
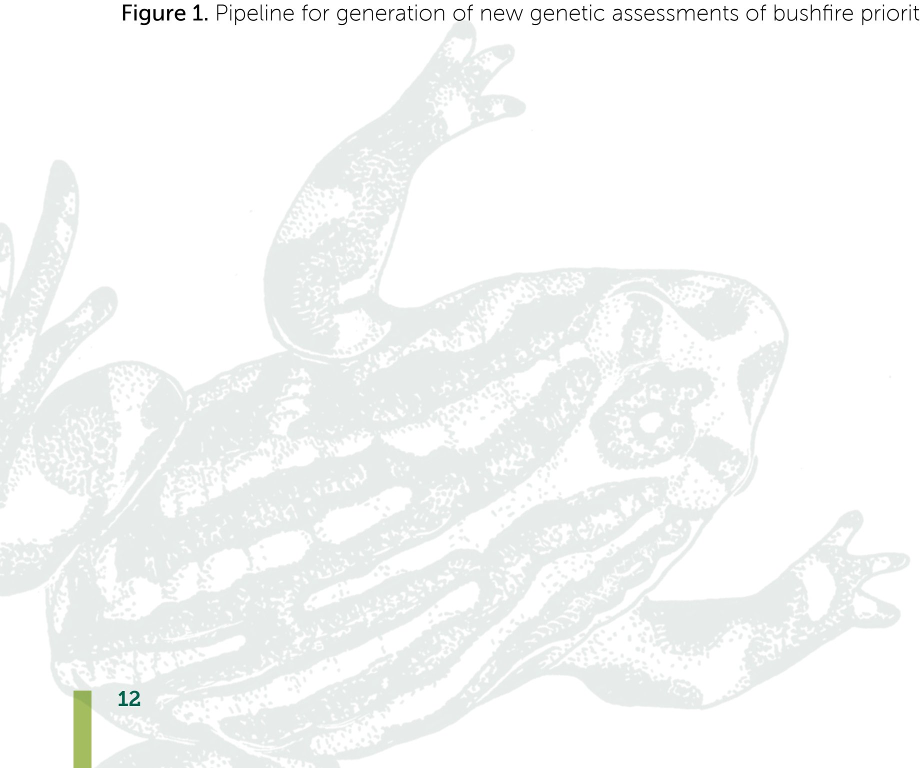


Figure 1. Pipeline for generation of new genetic assessments of bushfire priority species.



Application and impact of research

This project has led to an improved understanding of taxonomic and genetic diversity across high-priority groups in eastern Australia and supported the reassessment of federal threat listing status for multiple taxa by the Department of Agriculture, Water and the Environment (DAWE). As species were considered regardless of formal taxonomic status, the work completed here reduces the likelihood of cryptic extinction, or the loss of unknown species.

A major application of this project has been the incorporation of genetic structure within species into policy and statutory documents. This data is available for use when developing conservation advices at the federal level, and for state and non-government agencies to use when designing and prioritising conservation management actions. Using the genetic information developed during this project will lead to improved persistence of species over the longer term. The creation of detailed landscape genetic datasets will also provide an important baseline for the management of individual species, and benchmarking against future catastrophic events.

This project has provided the identification of areas and species where existing genetic sampling was too poor for assessment, enabling targeted additional sequencing and fieldwork. This knowledge has been applied by providing it to contractors carrying out post-bushfire ecological survey work. Tissue samples provided by these contractors will be incorporated into the future landscape genetic assessments. These data will help resolve taxonomic questions and conservation genetics issues for these threatened taxa. Samples not used in this project will improve our ability to assess the impacts of future disturbance, including bushfires, on genetic diversity in priority species.

Our future work analysing the spatial distribution of genetic diversity across the landscape will help guide government and non-government organisations in prioritising areas for conservation actions. This will enable agencies to maximise conservation outcomes while minimising the use of resources.

Broader implications

The extensive new genetic information generated in this project is a significant leap forward in genetic benchmarking priority species prior to future catastrophes. Genetic benchmarking enables before and after comparisons that provide information on the scale of impact of catastrophic events, or changes in species over time. These data are the largest co-distributed set of landscape genetic data in Australia. They will provide an important benchmark to assess changes in genetic diversity through time across threatened species and habitats.

We also identify key actions to improve the ability to rapidly benchmark biodiversity before and after future catastrophic events (Catullo et al. 2021). These include undertaking targeted tissue sampling of threatened species from key areas identified as lacking sampling, following the best practice for providing genetic information for use in policy and statutory documents including conservation advice (as discussed above), and developing an expanded taxonomic workforce to enable these rapid analyses.

Lessons from the workshop on how to best design and communicate genetic studies to conservation managers have applicability to all species, and the breadth of attendance at the workshop provides opportunities for future engagement. This project has highlighted the need for a more reliable system for connecting researchers and managers, as well as tracking the availability of genetic data for threatened species.

Future research priorities

The main challenges and issues to be addressed moving forward are:

1. **Understanding the spatial distribution of genetic diversity.** Understanding the spatial distribution of genetic diversity across the landscape for multiple species will allow managers to maximise conservation outcomes while minimising costs.
2. **Understanding how fire will impact diversity.** This project has developed knowledge of genetic diversity in priority species. Combining this with work from project 8.3.2, *Effect of fire severity on the response of populations of priority wildlife species*, will enable an understanding of how fire severity and management actions will impact genetic diversity over ten years.

Data sets

Landscape genomic data for approximately 50 species will be available through the BioPlatforms Australia data portal (https://data.bioplatforms.com/), following an initial embargo period.

Recommendations

The key recommendations from this project are:

1. **Incorporate genetic information into listings.** Conservation assessments should consider genetic information when listing matters under legislation. This includes considering taxonomic changes, and considering genetically distinct populations within species ranges as important populations.
2. **Genetic diversity informed conservation management.** Conservation advice and state-level management plans should incorporate information on the distribution of genetic diversity into management plans and actions, whenever possible. These data can improve the long-term persistence of the species by maintaining genetic diversity, identifying populations most important to the species, and populations that are relevant under future climates.
3. **Ongoing dialogue between managers, policy makers and geneticists.** This project has highlighted the need for ongoing dialogue between conservation managers, those writing policy and statutory documents, and geneticists. This will ensure that the information in policy and statutory documents is presented accurately, and that the outcomes and limitations of this information are understood.

Glossary

Admixture - mixing of genetic diversity between two genetically distinct groups.

Clade - a group of organisms that have descended from a common ancestor.

Evolutionarily Significant Unit (ESU) - historically isolated set of populations that are reciprocally monophyletic and show significant divergence.

Genetic drift - random fluctuations in the frequency of alleles between generations due to chance events.

Important population - defined under the *Environment Protection and Biodiversity Act 1999* as a population within a species listed as vulnerable that is necessary for a species' long-term survival and recovery.

Inbreeding depression - reduced individual fitness that occurs in small populations due to mating by related individuals.

Morphological - relating to the appearance, or morphology, of a species, including its size, shape and structure.

Acknowledgements

This project was supported by BioPlatforms Australia and the ANU Centre for Biodiversity Analysis.

Ethics statement:

All genomic work carried out within this project did not require animal ethics. Any collections undertaken by associated researchers were undertaken using appropriate approvals from their institutions.

References

Catullo, R.A., Schembri, R., Tedeschi, L.G., Eldridge, M.D.B, Joseph, L. and Moritz, C.C. (2021) 'Benchmarking taxonomic and genetic diversity after the fact: lessons learned from the catastrophic 2019-2020 Australian Bushfires'. *Frontiers in Ecology and Evolution*, 9: 292. doi: 10.3389/fevo.2021.645820

Chapple, D.G., Hoskin, C.J., Chapple, S.N.J. and Thompson, M.B. (2011) 'Phylogeographic divergence in the widespread delicate skink (Lampropholis delicata) corresponds to dry habitat barriers in eastern Australia', *BMC Evolutionary Biology*, 11. doi: 10.1186/1471-2148-11-191.

Coates, D. J., Byrne, M. and Moritz, C. (2018) 'Genetic Diversity and Conservation Units: Dealing With the SpeciesPopulation Continuum in the Age of Genomics', *Frontiers in Ecology and Evolution*, 6. doi: 10.3389/fevo.2018.00165.

Kriesner, P. et al. (2020) *Assessing genetic risks to Victorian flora and fauna*. Department of Environment, Land, Water and Planning. Available at: https://www.environment.vic.gov.au/__data/assets/pdf_file/0029/518492/Genetic-Risk-Index-Report.pdf

Legge, S. et al. (2020) *Rapid analysis of impacts of the 2019-20 fires on animal species, and prioritisation of species for management response*. Department of Agriculture, Water and the Environment. Available at: http://www.environment.gov.au/system/files/pages/ef3f5ebd-faec-4c0c-9ea9-b7dfd9446cb1/files/assessments-species-vulnerability-fireimpacts-14032020.pdf.

Moritz, C. (1994) 'Defining "Evolutionarily Significant Units" for conservation', *Trends in Ecology & Evolution*, 9(10), pp. 373–375. doi: 10.1016/0169-5347(94)90057-4.

Moritz, C. (1999) 'Conservation Units and Translocations: Strategies for Conserving Evolutionary Processes', *Hereditas*, 130(3), pp. 217-228. doi: 10.1111/j.1601-5223.1999.00217.x.

Weeks, A. R. et al. (2017) 'Genetic rescue increases fitness and aids rapid recovery of an endangered marsupial population', *Nature Communications*, 8(1), p. 1071. doi: 10.1038/s41467-017-01182-3.

Appendix

Genetic assessment of *Assa darlingtoni* Hip-pocket Frog/Marsupial Frog/Pouched frog

Species experts

Prof. Steve Donnellan (University of Adelaide)

Prof. Michael Mahony (University of Newcastle)

Description

A recent taxonomic revision of the Hip-pocket Frog (*Assa darlingtoni*) was undertaken using extensive genetic sampling (Mahony et al. accepted). Mahony et al (accepted) recognised two species, and this genetic assessment refers to the widespread species that continues to carry the name *A. darlingtoni*.

Distribution and habitat

The Hip-pocket Frog is a montane leaf-litter species distributed from the Sunshine Coast hinterland in Queensland through to Dorrigo Plateau in New South Wales. Geographically, there are five distinct subpopulations where the species is naturally present (Fig. 1, yellow). There is likely little to no movement and gene flow between each of the five separate subpopulations. This fragmentation has likely predated European occupation, and there are few signs of decline across the subpopulations (Mahony et al. accepted).

Mahony et al. (accepted) found *A. darlingtoni* to be eligible for listing as Endangered under IUCN criterion B2(a,b), as it has an area of occupancy of less than 500 km^2 and is severely fragmented. At the level of individual subpopulations, two are ranked as Critically Endangered (Conondale/Blackall Ranges, D'Aguilar) [B2(a,b)], and three as Endangered (MacPherson Ranges, Dorrigo Plateau and Gibraltar Range/Washpool) [B2(a,b)].

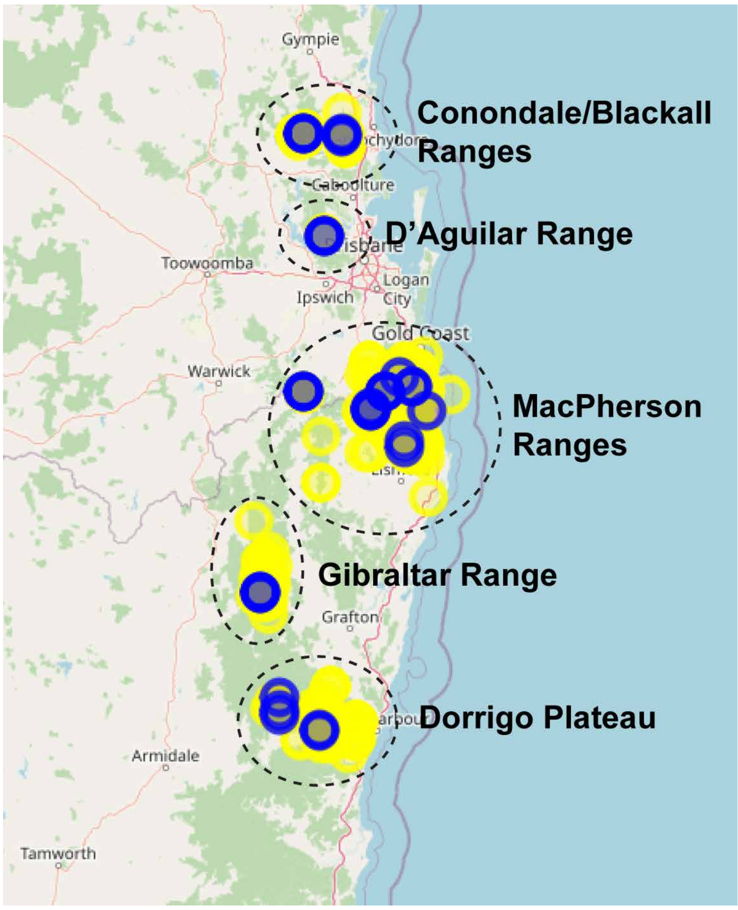


Figure 1. Distribution of *Assa darlingtoni* based on Atlas of Living Australia records (yellow). Samples used in genetic analyses are shown in blue. Outlines define the five geographically distinct subpopulations discussed in the text.

Genetic samples cover the current range of the species (Fig. 1, blue). The genetic evidence supports three major distinct genetic groups within the Hip-Pocket Frog (Fig. 2). The groups are the Conondale/Blackall Ranges and the D'Aguilar Range (Assa1), the eastern MacPherson Ranges plus the Gibraltar Range (Assa2), and in the western MacPhersons Range plus the Dorrigo Plateau (Assa3). All three groups show some genetic admixture in the MacPherson Ranges region, likely due to connectivity prior to european occupation. These data support that *A. darlingtoni* is a single geographically structured species.

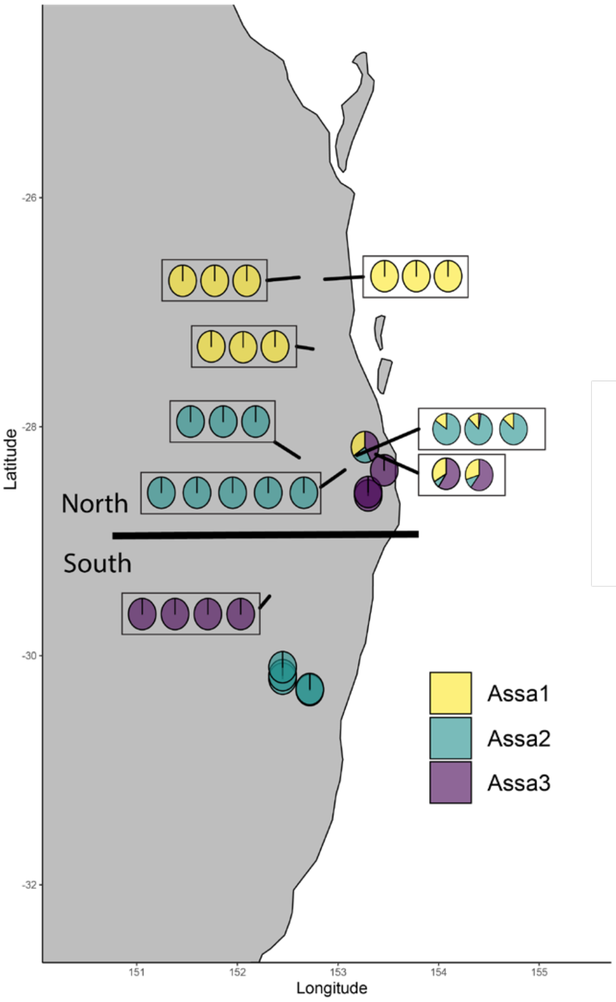


Figure 2. A map showing the distribution of genetic clusters within *A. darlingtoni*. Each individual is represented by a pie chart. The colours represent the genetic cluster to which an individual is assigned. Where individuals have multiple colours, that individual is genetically a mixture of different groups. For *A. darlingtoni*, the region around the MacPherson Ranges shows genetic mixing between the three genetic groups. The North/South boundary shows the dividing line between the north and south genetic groups of both Assa2 and Assa3.

Further genetic substructure is found in both southern groups (Assa2 and Assa3). For these groups, there is a distinct genetic break found between their northern and southern locations (Fig. 2, black line). This substructure is likely due to genuine gaps in the distribution. The southern lineage of Assa2 is likely restricted to the Gibraltar Ranges/Washpool region. The southern lineage of Assa3 is likely restricted to the Dorrigo Plateau. Genetic differentiation has not been identified within the two geographically separated groups within Assa1 (the D'Aguilar Range and Conondate/Blackall Ranges).

Genetic assessment of subpopulations

Assessments of genetic diversity within *A. darlingtoni* are based on further assessments of the single nucleotide polymorphisms used in Mahony et al. (accepted). Genetic diversity varies substantially across the distribution of the Hip-pocket Frog. For the purposes of this assessment, information about genetic diversity is assessed across each of the five geographically isolated subpopulations.

The most genetically diverse and geographically largest subpopulation is distributed across the MacPherson Ranges, including the Border Ranges and Mount Ballow (Mt Barney National Park) (Fig. 3). This subpopulation shows genetic admixture of all three genetic groups in Springbrook, Numinbah, and northern end of Lamington National Parks. The MacPherson Ranges subpopulation shows geographic structure that is important to conservation. In particular, there is an east/west divide that separates the Assa2 and Assa3 genetic clusters. The Assa2 group is present in Lamington and Mount Barney National Parks, and the Assa3 group is present in Mooball and Nightcap National Parks. Protecting the breadth of genetic diversity in the species will require conservation across the whole geographic range of this subpopulation.

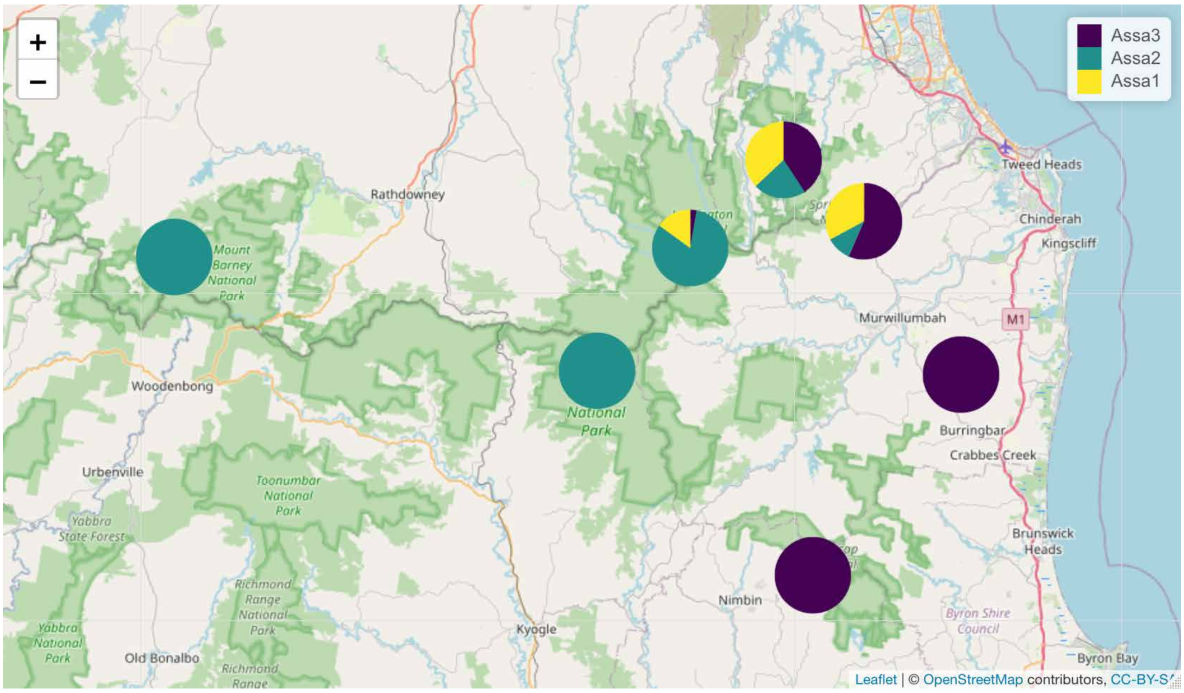


Figure 3. Distribution of genetic diversity across the MacPherson Range subpopulation. Each pie chart represents the portion of genetic diversity from that location that comes from each of the three major genetic clusters.

Genetic diversity is lowest at the extremes of the species range. Diversity was evaluated using heterozygosity - a measure that relates to risk of inbreeding depression and is often reduced in small and declining populations. When large numbers of individuals have high heterozygosity, this suggests high genetic diversity, random mating, and larger population sizes. Heterozygosity was substantially higher in the MacPherson Range subpopulation than in all other subpopulations (0.11 *versus* <0.05). This result indicates the MacPherson Range subpopulation is substantially genetically healthier than other populations.

Private alleles provide information on the uniqueness of genetic diversity in one population relative to others. A private allele is an allele that is present in one population, but not the other. An assessment of private alleles in the MacPherson Ranges relative to all other subpopulations identified over four times as many private alleles in the MacPherson Ranges (Table 1). When compared to each individual subpopulation, the number of private alleles in the MacPherson Ranges is increased to over 10 times that of each individual subpopulation. These data suggest there is only a small amount of genetic diversity within the species that is not present in the MacPherson Range subpopulation. Low numbers of private alleles distinguish the Conondale Range from the D'Aguilar Range, indicating low genetic differentiation.

Table 1. Number of private alleles identified in pairwise comparisons between subpopulations. High numbers of private alleles in one subpopulation relative to another indicate a high level of unique genetic diversity. Where one population is high and another low, this indicates most diversity within the low population is present in the high population. When both populations have high values, both are distinct.

Subpopulation 1 *versus* Subpop. 2		Private alleles in Subpop. 1 *versus* Subpop. 2	
MacPherson (n = 17)	All others (n = 17)	4742	1152
MacPherson (n = 17)	Conondale (n = 5)	6389	371
MacPherson (n = 17)	D'Aguilar (n = 3)	6610	299
MacPherson (n = 17)	Dorrigo (n = 5)	6594	307
MacPherson (n = 17)	Gibraltar (n = 4)	6463	287
Conondale (n = 5)	D'Aguilar (n = 3)	681	297
Conondale (n = 5)	Dorrigo (n = 5)	2185	1916
Conondale (n = 5)	Gibraltar (n = 4)	2022	1864
D'Aguilar (n = 3)	Dorrigo (n = 5)	1945	2062
D'Aguilar (n = 3)	Gibraltar (n = 4)	1782	2012
Dorrigo (n = 5)	Gibraltar (n = 4)	2325	2436

Threats

Climate change driving loss of suitable habitat is predicted to be a major threat to *Assa* (Keith et al. 2014). The model prediction is that the distribution of *A. darlingtoni* will contract to the cooler and moist uplands as climate warms, thus reducing the extent of the lower altitude distribution of each subpopulation. There are a number of lower altitude populations around the Tweed Caldera that may be adapted to warmer climates. However, it is currently unknown whether these populations are *A. darlingtoni*, or the new *Assa* species. Molecular genetic identification of these populations would be a priority as they exist between the distribution of *A. darlingtoni* and the newly described species. Consideration of translocation of individuals from warmer climates (low-lands and northern subpopulations) into cooler areas should be considered to improve the probability of persistence.

There is currently no empirical evidence of species-level decline, although this may change as climatically suitable habitats contract. The low genetic diversity in the extreme north and south of the species' range may indicate that these genetic groups will be more susceptible to inbreeding depression if a decline should occur. If declines are detected, regular monitoring and early intervention should be considered.

Recommendations

- Identification of the *Assa* species present in the lowland regions around the Tweed Caldera should be undertaken. If the lowland individuals are *A. darlingtoni*, translocations from the lowlands to higher elevation areas should be considered for the purposes of introducing climate-adapted diversity into cooler areas.
- Preservation of the MacPherson Range subpopulation, across the full distribution of the subpopulation, should e a priority for maintaining the genetic diversity of the species.
- Regular monitoring is needed to detect population declines as ranges contract.
- If translocations are required in the future to maintain diversity (noting that further genetic analyses of existing data should be undertaken prior to translocation actions to confirm suitability):
    - Genetic diversity in the MacPherson Ranges includes most diversity seen across all populations, and therefore this subpopulation is a suitable source for translocations to all other areas.
    - The Conondale and D'Aguilar Ranges subpopulations have low genetic diversity, and are genetically similar. Translocations between the two populations would be suitable. Translocations from the MacPherson Ranges subpopulation would be suitable, preferably from Springbrook, Numinbah, and northern end of Lamington National Parks. Translocations from the two southern subpopulations should be approached with caution as they may be genetically adapted to a cooler environment, but should not be ruled out if necessary.
    - The Gibraltar Range subpopulation has low genetic diversity. Translocations from the MacPherson Ranges would be most suitable (preferably from Lamington, Border Ranges, and Mount Barney National Parks), followed by the Dorrigo Plateau. Translocations from the two northern subpopulations may be considered for climate adaptation as discussed above.
    - The Dorrigo Plateau subpopulation has low genetic diversity. Translocations from the MacPherson Ranges would be most suitable (preferably from Numinbah, Mooball and Nightcap National Parks), followed by the Gibraltar Range. Translocations from the two northern subpopulations may be considered for climate adaptation as discussed above.

Research priorities

Research priorities that would inform future regional and local priority actions include:

- Further genetic sampling and research is required in the lowlands around the Tweed Caldera, to determine the range limits of the Hip-pocket Frog and the new *Assa* sp.
- Low-altitude and northern genetic groups (Conondale and D'Aguilar Ranges) may represent populations adapted to the climatic margins of the species. Research assessing the physiological and genetic adaptations of the individuals from these regions could support future climate-adjusted translocations to southern subpopulations.

Further information:

http://www.nespthreatenedspecies.edu.au

This project is supported through funding from the
Australian Government's National Environmental Science Program.

Threatened Species Recovery Hub

National **Environmental Science** Programme