Science for Saving Species

Research findings factsheet

Project 4.1.8

Threatened Species Recovery Hub

National **Environmental Science** Programme

Understanding genomic variation in the western ringtail possum and its application to effective conservation management

In brief

The habitat of the Critically Endangered western ringtail possum (*Pseudocheirus occidentalis*) has been greatly reduced and fragmented over time. There is a lack of information on small, remote populations of this possum, especially on the genetic diversity of isolated populations. To address these knowledge gaps, we generated thousands of genome-wide markers using next generation sequencing across the remnant range of the species. We used historical collections and opportunistically collected samples, to obtain genetic data for 305 samples across 23,146 loci.

Overall, the three current management zones for this species explained the genetic diversity within the species relatively well, with some exceptions. We found evidence for eight genetically defined subregions - four population clusters in the Swan Coastal Plain, two contemporary clusters in the Southern Forest and two clusters in the South Coast. As three of the four genetic clusters in the Swan Coastal Plain management zone are not in the Swan Coastal Plain, we propose this management zone be re-named to West Coast.

The Upper Warren population had high genetic diversity, and more conservation resources should be directed to this area to prevent substantial and irreversible loss of this genetic diversity, especially as the population is declining. Inbreeding could have occurred in populations at Albany City and Manjimup, and further monitoring is required to see if inbreeding depression has occurred.

The findings from this work will contribute to recovery objectives for the western ringtail possum and assist with translocations for the genetic management of this species.

Western ringtail possum in a jarrah tree (Eucalyptus marginata). *Image: Adrian Wayne*

THE UNIVERSITY OF WESTERN AUSTRALIA

GOVERNMENT OF WESTERN AUSTRALIA

Department of **Biodiversity, Conservation and Attractions**

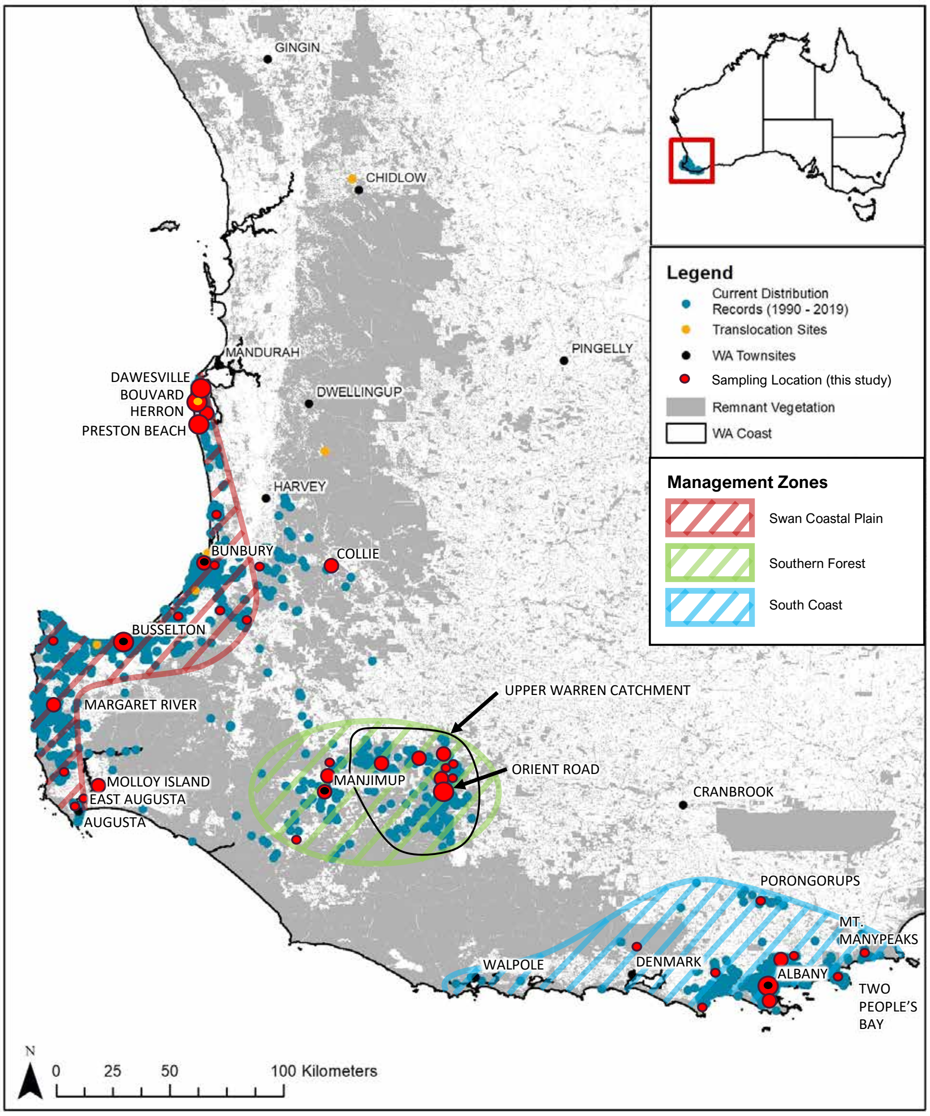
Background

The western ringtail possum or ngwayir (pronounced n-wa-yer) is an arboreal, nocturnal marsupial endemic to the south-west of Australia (Figure 1). Since European arrival their range has contracted, and their habitat is now reduced and fragmented as a direct result of human development. Current total census size is around 20,000 animals.

Since 1991, translocations of displaced or rehabilitated possums to eight approved sites have been attempted, however populations persist at just three locations – Karakamia Sanctuary, Perup Sanctuary and Yalgorup National Park. As a result of recent large population declines, the conservation status of ngwayir has been increased to Critically Endangered under the International Union for Conservation of Nature (IUCN) and the Environment Protection and Biodiversity Conservation (EPBC) Act.

In 2017 the Western Australia Department of Parks and Wildlife released the Western Ringtail Possum Recovery Plan, designed to guide recovery of ngwayir over the next 10 years. The plan identifies knowledge gaps including a lack of information for small, isolated, or remote populations. Historically, ngwayir have had focal management centres, especially in the southern Swan Coastal Plain. However, there remains a lack of information on the genetic diversity across the species. Genetic diversity is important for threatened species as high levels are associated with greater fitness and adaptive potential of populations. In contrast, small, isolated populations are susceptible to loss of genetic variation by genetic drift and often suffer inbreeding depression.

Figure 1. Contemporary distribution of ngwayir showing the three management zones, major landmarks, historical translocation sites and sampling locations for this study. Size of red filled circle indicates the number of samples from this location. Smallest < 5, 5 ≤ medium < 10, largest ≥ 10. Image adapted from WA Department of Parks and Wildlife (2017).



A 2009 study limited to part of the Swan Coastal Plain management zone and Southern Forest management zone (Figure 1) identified three genetically distinct populations – two in the southern Swan Coastal Plain (Gelorup and Busselton), which were both separate from the Upper Warren population.

Main aims of research

We first aimed to assess levels of genetic diversity in ngwayir populations, identify genetic clusters and estimate the relationships between these clusters. We then wanted to associate our genetic clusters with environmental variables so that environmentally distinct regions that sustain unique genetic populations could be identified. Ultimately, our objective was to increase the number of locations for which we have genetic data and identify ngwayir populations of high conservation priority and value.

Western ringtail possum in an Australian peppermint tree (Agonis flexuosa). *Image: Alan Danks*

What we did

This research was undertaken working in collaboration with the Department of Biodiversity Conservation and Attractions, and after consultation with the Western Ringtail Possum Recovery team and South Coast Western Ringtail Possum Working Group.

Ngwayir tissue samples for genetic analysis were collected by accessing historical collections, largely facilitated by DBCA. Gaps in the sampling landscape were filled with opportunistically collected samples, which was dependent on assistance from third parties including regional DBCA offices, wildlife carers and the Margaret River Natural Resource Management group. At project completion the database contained records for 515 samples from 57 locations, collected between 1997 and 2020.

Samples were prepared for genotype-by-sequencing, a ‘next generation’ sequencing technique which generates up to hundreds of thousands of genome-wide genetic markers called single nucleotide polymorphisms (SNPs). Sequencing and SNP genotyping was done on 325 samples. After removal of duplicates and failures, 23,146 SNPs from 305 samples and 52 locations were available for processing.

After quality checking and filtering data we selected the SNP markers most useful for our analyses, which included quantifying population diversity levels, defining genetic populations and describing relationships between populations.

We determined the number of operational taxonomic units (OTUs) by running fixed difference analysis on the genetic clusters. This type of analysis attempts to amalgamate populations that do not have fixed differences in alleles, so that individuals in an operational taxonomic unit share alleles that are absent in others, a strong indication of an absence of gene flow and sharing of genetic material.

To determine whether there is association between genetic and geographic distance all samples that had GPS coordinates were included in an isolation-by-distance analysis, using locations that had two or more individuals.

Finally, working with geospatial data analyst Stanley Mastrantonis, we explored whether there is any association between environmental variables and ngwayir genetic clusters. We selected the 14 most informative independent variables after collating geospatial data for 34 environmental variables including mean summer rainfall, mean summer temperature, maximum summer temperature and elevation.

What we found

Genetic diversity was estimated from a dataset that consisted of samples from 247 ngwayir and 5894 loci. Genetic diversity was greatest in eight locations within the Southern Forest management zone (Figure 1).

Inbreeding coefficient values range from -1 to 1, where negative values indicate greater heterozygosity than expected, and positive values indicate less than expected, indicative of inbreeding. Inbreeding could have occurred in populations at Albany City (F_{IS}=0.118 ± 0.004) and Manjimup (F_{IS}=0.162 ± 0.005). More monitoring is required for these populations to see if inbreeding depression (e.g. higher mortality, lower fecundity, physical abnormalities) has occurred.

The number of private alleles in a population is the number of alleles that are unique and are not shared with any other population. There was a low number of private alleles per sample in the greater Albany locations (e.g. 0.5 in Albany King River, 3.0 in Albany City). This was expected due to their close geographic proximity, meaning these populations are probably interbreeding and freely sharing genetic material. The largest number of private alleles was found in samples from Molloy Island (17.0), Pemberton (17.5) and Two People's Bay (18.7). These high values reflect the low geographic connectivity between these populations and all other populations (Figure 1), and show these populations have unique allelic (genetic) diversity.

Genetic clusters and management zones

In general, the three ngwayir management zones as currently described, explain species-wide genetic diversity relatively well, with some important expansions. We found evidence for four population clusters in the Swan Coastal Plain, two contemporary clusters in the Southern Forest and two clearly defined clusters in the South Coast. Details of the eight genetic clusters can be found in Table 1. Based on the identification of four genetic clusters in the Swan Coastal Plain management zone, three of which are not in the Swan Coastal Plain, we propose this management zone be re-named to West Coast (Table 2).

Genetic diversity statistics for each cluster showed the population at upper Warren to have the greatest expected heterozygosity (He= 0.078 ± 0.001) and allelic diversity (Na=1.70 ± 0.006, mean number alleles per marker). The maximum allelic diversity possible using this measure is 2.0, indicating that the Upper Warren population has a high level of genetic diversity. The lowest expected heterozygosity (He= 0.049 ± 0.002) and allelic diversity (Na= 1.38 ± 0.006), was found in the Southern Swan Coastal Plain, when examining locations with 5 or more samples.

Pairwise genetic distance analysis showed that the Two People's Bay cluster had the highest genetic distance values, especially in relation to the Southern Swan Coastal Plain, Capes Region and Lower Blackwood River Catchment clusters (Table 3). This result could be affected by the low sample size for the Two People's Bay cluster, which can lead to overestimates of genetic distance. Nonetheless, distance results combined with private allele analysis highlights how genetically distinct the possum population at Two People's Bay may be. The geographic isolation of Two People's Bay could create a barrier to gene flow resulting in this pattern. Interestingly, the Lower Blackwood River cluster was genetically less distant from the Upper Donnelly River Catchment and Upper Warren, than to its geographically closest cluster, the Capes Region, suggesting the Blackwood River may act as a barrier to gene flow between the Lower Blackwood River and Capes Region subregions.

When looking at all the data, and as expected, there was a significant positive relationship between geographic location and genetic distance. In general, the more geographically distant populations are from each other, the less gene flow that has occurred between them.

We only identified one operational taxonomic unit, which encompassed all subregions, suggesting all ngwayir are one interconnected metapopulation. However, when the frequency threshold of a fixed allele difference was relaxed in our analysis, three operational taxonomic units were identified – Two People's Bay, Lower Blackwood River Catchment, and a larger unit that amalgamated all other genetic clusters. Although this signal may be inflated by the small sample size for the Two People's Bay and Lower Blackwood River Catchment subregions, this trend reinforces our other findings that there is limited gene flow between parts of the ngwayir population in Western Australia, and that they have genetic differences but not enough to be classified as separate subspecies.

Mean summer rainfall was the most important environmental variable for predicting assignment of possums to genetic clusters (Figure 2). Mean summer temperature and the evapotranspiration rate in February were the second and third most important environmental predictor variables, respectively.

What we found (continued)

Table 1. The genetic clusters we identified, the locations included in each cluster and the subregion name.

Cluster number	Number of individuals	Locations included in cluster	Subregion name
1	12	Augusta, Margaret River, Yallingup	Capes Region
2	18	Bunbury, Boyanup, Collie, Dalyellup, Donybrook, Gelorup, Leschenault, Picton	Leschenault Catchment
3	88	Bouvard, Busselton, Dawesville, Herron, Lake Clifton, Myalup, Preston Beach	Southern Swan Coastal Plain
4	5	East Augusta, Molloy Island	Lower Blackwood River Catchment
5	18	Alco, Manjimup, Palgarup	Upper Donnelly River Catchment
6	55	Camelar, Corbal, Keninup, Kingston National Park, Mordalup, Orient Road, Pemberton, Talling, Yackelup, Yendicup	Upper Warren
7	41	Albany and suburbs, Goode Beach, Mount Lindesay	Greater Albany
8	4	Mount Manypeaks, Two People's Bay	Two People's Bay

Table 2. The new management zone names and subregions assigned to each zone based on our genetic analysis.

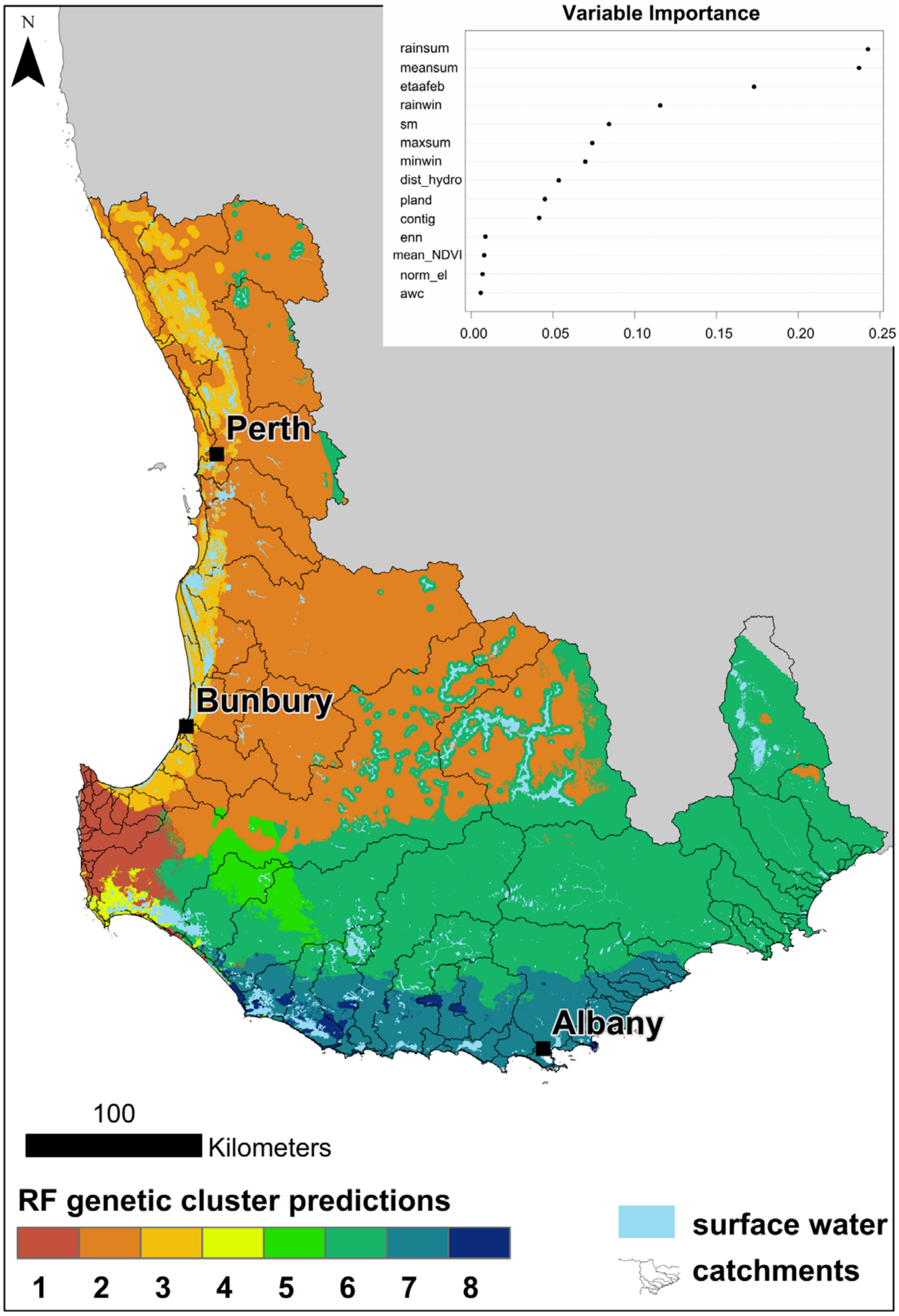
Old Management Zone	New Management Zone	Assigned subregions
Swan Coastal Plain	West Coast	Capes Region, Leschenault Catchment, Southern Swan Coastal Plain, Lower Blackwood River Catchment
Southern Forest	Southern Forest	Upper Donnelly River Catchment, Upper Warren
South Coast	South Coast	Greater Albany, Two People's Bay

Table 3. Pairwise comparisons of genetic distance between each population cluster. All pairwise comparisons were statistically significant. The larger the number, the more genetically different the populations in each cluster are from each other. The upper triangle represents one measurement of genetic distance (Jost's D_{est}) and the lower triangle represents another (pairwise F_{ST}).

	Southern Swan Coastal Plain	Leschenault Catchment	Capes Region	Lower Blackwood River Catchment	Upper Donnelly River Catchment	Upper Warren	Greater Albany	Two People's Bay
Southern Swan Coastal Plain	0	0.06	0.05	0.19	0.13	0.11	0.15	0.23
Leschenault Catchment	0.09	0	0.07	0.15	0.09	0.06	0.12	0.17
Capes Region	0.08	0.08	0	0.16	0.10	0.09	0.13	0.20
Lower Blackwood River Catchment	0.27	0.17	0.19	0	0.15	0.12	0.18	0.25
Upper Donnelly River Catchment	0.18	0.10	0.11	0.15	0	0.04	0.08	0.15
Upper Warren	0.15	0.07	0.09	0.12	0.04	0	0.07	0.11
Greater Albany	0.21	0.14	0.16	0.21	0.10	0.08	0	0.13
Two People's Bay	0.30	0.17	0.22	0.24	0.14	0.10	0.15	0

What we found (continued)

Figure 2. Environmental variable predictors of genetic cluster membership, showing relative importance of variables. Mean summer rainfall (rainsum), mean summer temperature (meansum) actual evapotranspiration rate in February (etaafeb) were the most important environmental predictors.



Rainsum = mean summer rainfall, meansum= mean summer temperature, etaafeb =actual evapotranspiration rate in February, rainwin = mean winter rainfall, sm = soil moisture, maxsum= maximum summer temperature, minwin= minimum winter temperature, dist_hydro =distance to non-natural hydrological features, pland = percentage of landscape belonging to each class of landscape/habitat, equal to 100 when only one patch is present, contig = habitat contiguity, enn = Euclidean nearest-neighbor distance, mean_NDVI = mean normalised difference vegetation index, awc = actual water content of soil, norm_el = normalised elevation.

Implications for target audience

We have been able to document the genetic diversity within ngwayir, and confirm the unique genetic identity of established, geographically separated groups. We have expanded on this by describing novel, genetic variation of potentially high conservation value, specifically in two genetically distinct clusters that appear to be more distinct and less connected to the remainder of the species. Two People's Bay and the Lower Blackwood River catchment are extreme parts of this animals' distribution, and this is the first time they have been genetically characterised.

Our mapping of genetic variation on to environmental layers revealed that inclusion within distinct genetic clusters can be predicted by combinations of environmental variables. In a climate that is becoming hotter and drier, this will help understand environmental resilience within ngwayir and undoubtedly contribute to informed management decision-making.

We recommend that there is recognition of at least eight genetically distinct subregions within ngwayir and that they are considered as 'management units' in all conservation and recovery planning. We recommend that the Swan Coastal Plain management zone is re-named to West Coast.

Ongoing monitoring should be undertaken to determine if there are signs of inbreeding depression in the populations at Manjimup and urban Albany.

Conservation resources should be allocated to prevent substantial and irreversible loss of the high levels of genetic diversity observed in the Upper Warren population, especially as the population is in decline.

Efforts should also be made to prevent further loss of genetic diversity in the Southern Swan Coastal Plain subregion by informed mixing between locations and supplementation from other genetic clusters, such as Upper Warren. Before any possums are translocated between distinct genetic clusters, the cluster assignment, genetic diversity and associated environmental variable predictors of the source and recipient populations should be considered.

While all ngwayir can be considered one interconnected population, we recommend that increased sampling and further OTU analysis are done on the Lower Blackwood River and Two People's Bay subregions, and the Pemberton locality.

Two People's Bay, Western Australia. Image: Bl0de, Flickr, CC BY-NC-SA 2.0

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Male western ringtail possum in a heartleaf tree (Gastrolbium bilobum).
Image: Adrian Wayne

Further Information

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