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Adaptive Genetic Management of a Reintroduction Program from Captive Breeding to Metapopulation Management of an Arboreal Marsupial

Jennifer C. Pierson [1,2,3,*], Laurence Berry [4], Lauren Alexander [5,6], Jennifer Anson [1], Michelle Birkett [7], Leah Kemp [1,†], Bruce A. Pascoe [8], Katherine A. Farquharson [5,6] and Carolyn J. Hogg [5]

1 Australian Wildlife Conservancy, Subiaco East, WA 6008, Australia; jennifer.anson@australianwildlife.org (J.A.); leah@natureadvisory.com.au (L.K.)
2 Fenner School of Environment and Society, Australian National University, Acton, ACT 2601, Australia
3 Centre for Conservation Ecology and Genomics, Institute for Applied Ecology, University of Canberra, Bruce, ACT 2617, Australia
4 Department of Energy, Environment, and Climate Action, East Melbourne, VIC 3002, Australia; laurence.berry@delwp.vic.gov.au
5 School of Life & Environmental Sciences, The University of Sydney, Sydney, NSW 2006, Australia; lauren.alexander@sydney.edu.au (L.A.); katherine.farquharson@sydney.edu.au (K.A.F.); carolyn.hogg@sydney.edu.au (C.J.H.)
6 ARC Centre of Excellence for Innovations in Peptide & Protein Science, The University of Sydney, Sydney, NSW 2006, Australia
7 Zoos South Australia, Adelaide Zoo, Adelaide, SA 5000, Australia; mbirkett@zoossa.com.au
8 Alice Springs Desert Park, Department of Environment, Parks and Water Security, Alice Springs, NT 0870, Australia; bruce.pascoe@nt.gov.au
* Correspondence: jennifer.pierson@australianwildlife.org
† Current address: Nature Advisory Ptd Ltd., Hawthorn East, VIC 3123, Australia.

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Abstract: The application of genetic data to conservation management programs can be hindered by the mismatch in timelines for management decisions and the acquisition of genetic data, particularly genomic sequence data that may require outsourcing. While applying genetic principles where data are absent can provide general guidelines for actions, genetic data can often fine-tune actions through adaptive management. We describe the adaptive genetic management of the establishment of a metapopulation of a small arboreal marsupial, the red-tailed phascogale (*Phascogale calura*). Two captive breeding programs were established as source populations, with genetic principles applied to the establishment of the first program and empirical genetic data used to guide the establishment of the second program. Genetic data from both programs were then used to allocate founders to three new populations to create a metapopulation with diversity both within and among the sites. Building and maintaining the diversity of metapopulations when recovering threatened species will reduce pressure on the original source populations and increase the resilience of the species.

Keywords: translocation; genetic management; captive breeding; reintroduction; red-tailed phascogale; genetic diversity

1. Introduction

As biodiversity continues to decline, translocations are becoming an increasingly important tool to re-establish populations in places where they have become locally extinct [1–3]. Increasing the number of populations in a species decreases the overall probability of extinction by spreading the risk of impacts from stochastic and weather-related events. Re-establishing populations in regions of a species' historic distribution can conserve the adaptive capacity of species by exposing populations to a range of environmental selection pressures [4,5].

Despite decades of research on translocation biology [2], there remains inconsistent results in translocation success [1]. However, a common factor that contributes to successful translocations is a large number of founders [3,6], ideally from multiple source populations, to maximise genetic diversity [7,8]. While wild-to-wild translocations are favoured due to the challenges that can arise when using captive-bred individuals for translocations [9,10], some species require captive breeding as an interim measure to ensure the minimum number of individuals needed for the translocation are available. Captive breeding may be particularly important for species that are continuing to decline in the wild and do not have robust wild source populations. Captive breeding for release can also provide an avenue to ensure that the genetic diversity acquired during the wild capture is amplified and mixed prior to the founder group of animals being released.

Captive breeding for release remains a powerful tactic for conservation despite the concerns identified [10,11]. Breeding for release requires planning to ensure the founder population has sufficient numbers and genetic diversity to provide a strong foundation [12] and can produce the required number of individuals to maximise the chance of success when released to the wild [3]. A wealth of research on optimal captive breeding strategies has been undertaken [8,9,13,14], much of which has been integrated into practice [11]. However, there continue to be challenges in obtaining ideal outcomes, such as managing genetic diversity [15], due to practical limitations encountered, such as breeding success in captivity. There is also a disconnect between the intensive management required to maximise pairings and the need to incorporate natural processes to reduce adaptation to captivity as much as possible [16]. While both conventional pedigree management and molecular genetic data are considered complementary [15,17], the time required to obtain genetic data often means post hoc assessment is more tractable than the proactive use of genetic data in detailed breeding management. Additionally, practical considerations in the husbandry of animals, particularly when natural conditions are emulated with limited human contact, means that known pairings may not be possible.

Breeding-for-release programs that are integrated with planned reintroductions may have specific objectives that influence the genetic management of captive breeding [18]. For example, locally adapted individuals may be desired, and a breeding program could be founded with individuals from an environmentally similar source location compared to the targeted reintroduction site. Alternatively, maximising overall genetic diversity through mixing several source populations may be an objective to maximise adaptive capacity. Capturing the remnant diversity available in the wild for the conservation of genetic diversity itself may be yet another objective. Identifying the specific objectives of the reintroduction and breeding programs a priori can facilitate the planning, and adaptive management of programs, as they can be evaluated along the way to direct ongoing management [19]. While adaptive management—the use of monitoring data to inform iterative decisions—has been a common approach in conservation for decades [20], incorporating genetic management into an adaptive management approach is more recent in conservation programs [21].

Here, we describe the adaptive genetic management of a breed-for-release program for the establishment of multiple red-tailed phascogale (*Phascogale calura*) populations. The red-tailed phascogale is an Australian endangered semi-arboreal carnivorous marsupial that has declined significantly over the last century and now occupies less than 1% of its former range [22]. They are a good candidate for wide-scale reintroduction efforts in areas where predation by feral cats and red foxes can be reduced [22] due to their wide-ranging use of vegetation types, with canopy density and tree hollow availability associated with their presence [23]. However, the small remaining population spread across fragmented patches makes them vulnerable to declines [22]. Therefore, a breeding program is needed to provide sufficient founders for multiple reintroductions without putting too much pressure on the wild remnant populations as source populations.

The red-tailed phascogale is a semelparous breeder, meaning there is an annual die-off of males after the breeding season and a low proportion of females that survive to a second year [24]. This annual die-off increases the risk of non-establishment of a new population,

particularly with a small number of founders. Implementing a breeding program has the potential to maximise the contribution of wild-caught individuals for species recovery by facilitating successful breeding and rearing of the high number of young produced per female (up to eight) and has the potential to provide individuals for supplemental releases if needed [24].

To help recover the species, the Australian Wildlife Conservancy (AWC) planned multiple translocations into fenced reserves across the species' former distribution and will manage the species as a metapopulation ongoing. In Australia, fenced reserves provide a 'safe haven' for species vulnerable to fox and cat predation to recover without the threat of predation, providing important source populations for future efforts to recover the species beyond the fence as well [25,26]. To increase the species' resilience to climate change, a key component of managing adaptive capacity is exposing the species to the range of former environmental conditions experienced prior to the population decline, as well as maintaining genetic diversity within and across sites.

AWC established two breeding programs in partnership with the Alice Springs Desert Park (ASDP) and Zoos South Australia (Zoos SA) to provide founders for the Newhaven Wildlife Sanctuary, Northern Territory; Mallee Cliffs National Park (NP) in New South Wales; and the Scotia Wildlife Sanctuary, New South Wales in Australia. Given the lack of prior genetic information in this species and uncertainty over the remnant population size, adaptive management was a key feature in developing a successful breed-for-release program to establish additional sites in the metapopulation, with over 100 founders desired for each site. The initial founders of the first breeding program were selected based on genetic principles but without empirical genetic data. The second phase of captive breeding was informed by genetic assessments of the wild and captive breeding outcomes. Genetic assessment of the second breeding program informed decisions on the allocation of founders to the fenced reserves to establish a metapopulation based on genetic assessments of both breeding programs. The projected population size at the Newhaven Sanctuary is 625, the Mallee Cliffs NP is projected to sustain up to 1700, and the Scotia Sanctuary is projected to sustain 1400; in combination, these populations will significantly increase the global population size and restore the species to portions of their range where they are locally extinct.

2. Materials and Methods

The adaptive management pathway required several iterative steps (Figure 1) to identify founders for both the captive breeding programs and the subsequent translocations. In brief, the first captive breeding program was established at ASDP in 2019 primarily as a source for Newhaven Sanctuary and to provide some founders for Mallee Cliffs NP. Subsequently, a second breeding program was established at Zoos SA in 2021 to primarily provide founders for Mallee Cliffs NP. The Zoos SA Program was then extended in anticipation of founding a population at Scotia Sanctuary. Initially, genetic data were not available for the wild population, and genetic principles were used to guide the founding of breeding programs by selecting sites that were geographically separated. Genetic data were obtained iteratively throughout the process, first on the wild individuals from different sites, then founders and offspring of the ASDP program, followed by the founders and offspring of the Zoos SA program. Logistical, regulatory, and ecological constraints were all balanced with the decisions targeting best-practice genetic management.

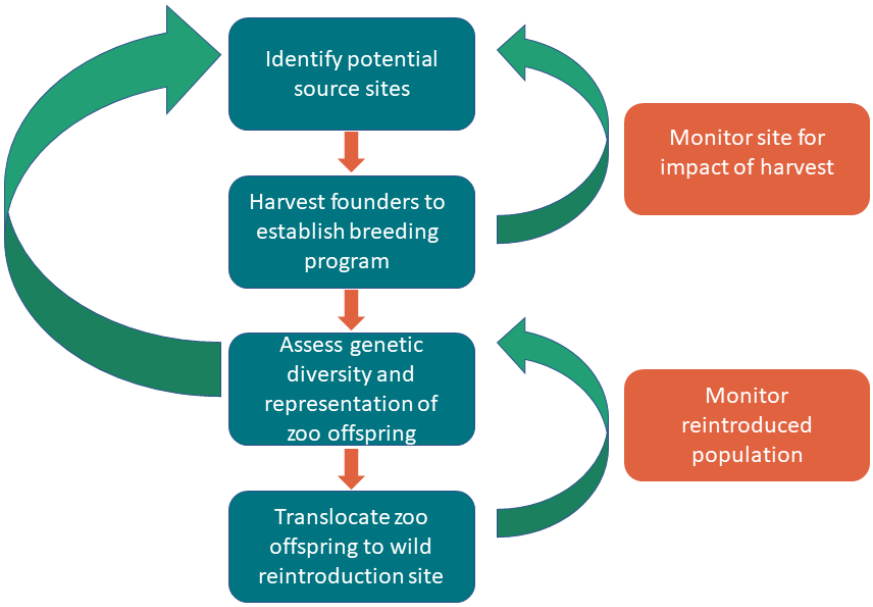

Figure 1. The adaptive genetic management pathway to establish a genetically diverse metapopulation. The adaptive management feedback loops are indicated by the green arrows.

2.1. Wild Source Population Assessment

The red-tailed phascogale is currently restricted to an area of fragmented remnant woodlands in South-west Western Australia (Figure 2). A total of 12 remnant sites were identified as potential sources to provide founders for each captive breeding program. Given the lack of genetic data for the wild remnants, sites were selected to harvest for the ASDP in 2019 on the basis of known or very likely populations of red-tailed phascogales, with larger patch sizes at greater geographic distances preferred on the basis that these sites would capture the greatest range of genetic variation across the metapopulation. Following adaptive management, the selection of sites to harvest individuals to establish the Zoos SA population in 2021 was informed by genetic analyses of samples collected during pre-translocation surveys in 2020 ASDP offspring. On this basis, candidate sites for the Zoos SA breeding program and NSW translocations were selected to maximise remnants under-represented in the ASDP breeding program.

Pre-translocation monitoring was conducted at each wild remnant site to determine the feasibility of translocating individuals from each remnant and assess the potential impacts of removing individuals from the populations. Initial surveys were undertaken in 2016 and 2017 as part of an earlier translocation program for Mount Gibson Sanctuary (AWC Sanctuary; Figure 2), in 2019 to establish the ASDP breeding program, and in 2020 and 2021 to establish the Zoos SA breeding program. Insufficient individuals were captured in 2020 to permit a translocation to occur. All surveys were conducted prior to the annual peak in breeding activity during May and April.

Within each remnant, surveys included two trapping grids, each comprising a total of 48 Elliott traps spaced 40 m apart. Ten cage traps were also deployed within the grid to reduce Elliott trap disturbance by Common Brushtail Possums (*Trichosurus vulpecula*). Traps were baited with peanut butter, oats, and sardines and set and checked over four nights. Morphometric and demographic measures and DNA samples (ear biopsies) were collected from all individuals captured during the surveys.

While potentially genetically desirable, some sites were deemed unsuitable for translocation due to low capture rates during pre-harvest surveys or ongoing research or land management programs.

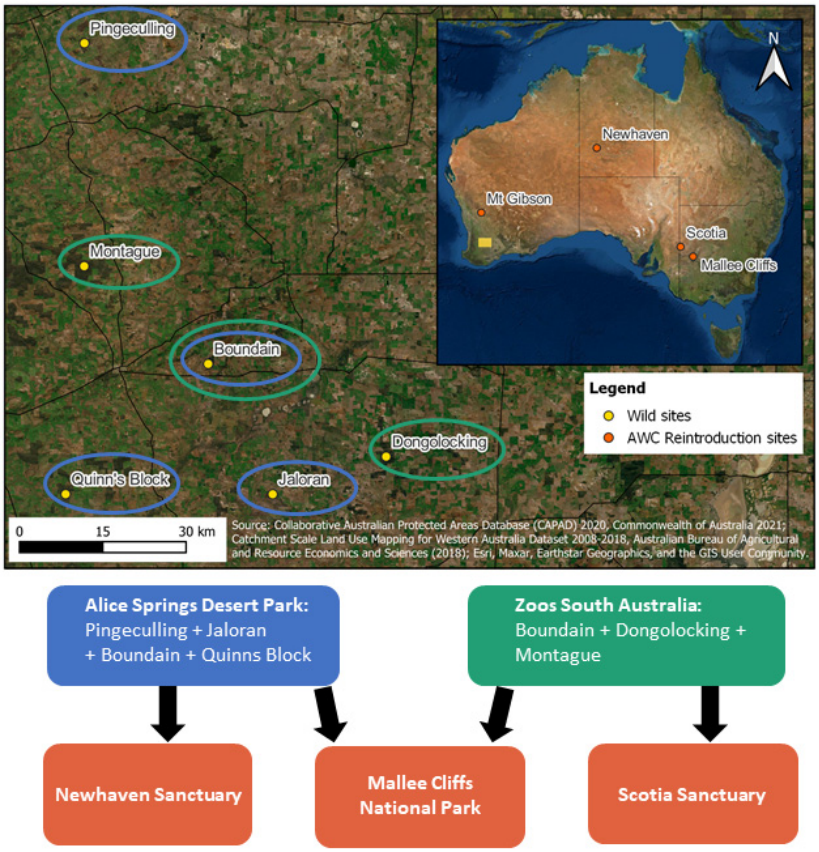

Figure 2. Locations of remnant sites (yellow rectangle is enlarged to show wild sites indicated with a yellow circle) that were assessed to determine suitability as source sites for red-tailed phascogale captive breeding programs. Australian Wildlife Conservancy's reintroduction sites are indicated with orange circles in the inset map of Australia. The source sites used for the two captive breeding programs are indicated with blue (ASDP) or green (Zoos SA) circles, with the mix of individuals used to found the different wild sites shown: Newhaven = 100% ASDP; Mallee Cliffs = 50/50 ASDP and Zoos SA; Scotia = 100% Zoos SA.

2.2. Translocation Methods

Individuals were sourced for captive breeding programs from sources sites that met the selection criteria following pre-translocation surveys. Subsequent trapping for translocation to ASDP in 2019 and Zoos SA in 2021 followed the same procedures. Trapping of animals occurred over one night and involved four grids of 48 Elliott traps at each site. Traps were wired open with the trapping mechanism disabled and pre-baited for three nights prior to the night of capture. Captured individuals were weighed and tagged with an identifying microchip and had an ear biopsy sample collected for genetic analysis. Founders were also given Richtasol and Hartmann's solution to reduce risks of capture myopathy and provided fluids for the duration of transport. Founders were each individually placed into wooden transport boxes filled with shredded paper, mealworms, and chopped carrots for transport. An ambient temp of 25 °C was maintained throughout the duration of both road and air transport. For translocation to the ASDP breeding program, founders were transferred to Perth airport via road and flown to Alice Springs (max 17 h travel); For translocation to Zoos SA, individuals were transferred via road to Narrogin airport following capture and flown to Adelaide (max 10 h travel).

2.3. Captive Breeding Program

The ASDP is located seven km west of Alice Springs, Australia, and is managed by the Northern Territory Government's Parks and Wildlife Commission. The ASDP breeding

program was founded in May 2019 with 21 individuals; however, 1 female from Pingeculling died after arrival, resulting in 20 founders to the program from four sites (Table 1a). Females were divided into three groups (5, 5, and 4) based on provenance, with group 1 consisting of five females from Pingeculling, Group 2 with four females from Boundain and one from Pingeculling, and Group 3 with two females from Jaloran and two from Quins Block. Animals were housed in 6 × 2 m enclosures, with males housed individually in smaller enclosures adjacent to the females. The males were rotated through the female groups from mid-May to late August, with rotations taking place twice weekly. In 2019, 41 offspring were produced, with 17 individuals retained for ongoing breeding. In 2020, female group size was increased to 6, and 99 young were weaned. In 2021, 86 young were bred from 14 females. The grouped nature of the housing did not facilitate location-specific breeding success estimates.

Table 1. (**a**) Founders of the ASDP Breeding Program in 2019. (**b**) Founders of the Zoos SA Breeding Program in 2021.

Source	Males	Females	Total
	(**a**)		
Pingeculling	3	7	10
Boundain	2	4	6
Jaloran	1	2	3
Quinns Block	0	2	2
Total	6	15	21
	(**b**)		
Boundain	2	10	12
Dongolocking	2	6	8
Montague	2	0	2
Total	6	16	22

The Zoos SA breeding program was founded in 2021 with 22 individuals (Table 1b) from three sites. The program followed a similar protocol to that applied by ASDP, with knowledge sharing actively occurring between facilities. In 2021, three enclosures with five to six females each were set up, and two males were rotated every three to four days. In 2022, twenty-one females (two of which were wild founders) were housed in six enclosures, with three to four females each, with two males (six males total) rotating every three to four days. In 2021, 24 offspring survived to adulthood, and 53 offspring survived in 2022. The grouped nature of the housing did not facilitate location-specific breeding success estimates.

2.4. Genetic Methods

2.4.1. DNA Extraction and Sequencing

DNA was extracted using a TNES salting-out method suitable for small tissue types. DNA concentration was quantified by Nanodrop (ThermoFisher), and fragmentation was assessed using 1% agarose gel electrophoresis at 90 V for 30 min. Extracted DNA was sent to Diversity Arrays Technology Pty Ltd. (Canberra, Australia) for library preparation and DArTseq, a form of reduced representation sequencing that generates single nucleotide polymorphism (SNP) data. Samples were sequenced as single-end 83–138 bp reads on a NovaSeq 6000 (Illumina) with the *PstI* and *NlaIII* restriction enzymes. A total of 54 samples were sequenced in replicate in order to calculate sequencing error rate.

2.4.2. SNP Calling

The raw FASTQ files were processed following the Stacks/R pipeline outlined in Wright et al. [27]. A Pawsey cloud-based machine was used to process genetic data (the Pawsey Supercomputing Research Centre, Perth, Australia; 64vCPUs, 256GB RAM, 3TB storage). Briefly, raw reads were cleaned with Stacks v2.53 'process_radtags' to remove barcodes and low-quality reads and truncate reads to 68 bp [28]. Adapter contamination was

removed using Trimmomatic v0.39 with the ILLUMINACLIP:Truseq3-SE:2:30:10 parameter, SLIDINGWINDOW:4:5, and LEADING:5 [29]. Cleaned, trimmed reads were aligned to the male brown antechinus (*Antechinus stuartii*) genome [30] with BWA aln [31]. Samtools v1.11 was used to sort and convert the alignments to BAM format [32]. A catalogue of variants across all samples was built with 'gstacks'; then, the 'populations' module was used to call SNPs with a minimum minor allele frequency (MAF) of 0.01, minimum proportion of samples genotyped (-r) of 0.3, and retaining one random SNP per tag. The VCF file was processed in R v4.1.2 [33] with the 'vcfR' package [34]. Only SNPs with an average allelic depth >2.5x, coverage difference between the reference and alternate allele $\leq$80%, reproducibility between technical replicate sequences $\geq$90%, call rate $\geq$80%, heterozygosity $\leq$70%, and a minor allele frequency of 0.01 were retained.

2.4.3. Population Genetic Analyses

Population genetic statistics were calculated for each of the six wild founding sites (Montague, Boundain, Dongolocking, Jaloran, Pingeculling, and Quinn's Block) and the offspring of the two captive sites (ASDP and Zoos SA). Average observed heterozygosity (H_O), expected heterozygosity (H_E), and their standard errors were calculated for each site using GenAlEx [35]. Standardised heterozygosity (H_S) across individuals was calculated with the 'genhet' package [36] in R. Inbreeding coefficients (F_{IS}) and the associated 95% confidence intervals were obtained using the 'diveRsity' package in R [37]. Allelic richness was calculated with the 'hierfstat' package in R [38].

Genetic differentiation between sites was visualised via principal coordinate analyses (PCoA) for (1) all samples, (2) for the ASDP offspring with the wild founding sites, and (3) for the Zoos SA offspring with the wild founding sites. The 'StAMPP' package [39] in R was used to estimate F_{ST}, a measure of genetic differentiation. Genetic clustering was investigated using fastSTRUCTURE v1.0 [40] with the same three data sets as the PCoAs. We tested varying the number of genetic clusters from K = 1 to K = 6, with the 'chooseK.py' script to determine the optimum number of clusters. The optimum value was used to create a STRUCTURE-style stacked bar graph with base R plotting functions.

Finally, we performed kinship analysis to estimate the pairwise relatedness of all individuals using the triodic maximum likelihood estimator (TrioML) in COANCESTRY v1.0.1.9 [41]. Relatedness estimates were halved to obtain the mean kinship (MK). MK provides an estimate of how related one individual is to the rest of the population. We examined the MK relationships between males and females in the captive breeding population to provide recommendations on pairings to prioritise or avoid. Pairs with low MK were prioritised for breeding.

3. Results

3.1. Wild Assessment and Harvest

Twelve sites were surveyed across five years, with a total of 218 individuals captured (Table 2). Sites were only surveyed if being considered as a source for a future translocation and were not monitored annually as part of an ongoing monitoring program.

In April 2019, a total of 21 individuals (6 males and 15 females) were harvested from Pingeculling, Boundain, Jaloran, and Quinn's block remnants to establish the captive breeding program at ASDP. In May 2021, a total of 22 individuals (6 males and 16 females) were collected from Boundain, Dongolocking and Montague sites to establish the captive breeding program at Zoos SA. While the threshold for take (n = 8) was not met at Montague, permission was granted on the basis that these individuals had the potential to improve genetic diversity within the captive breeding program. Dongolocking and Montague had also not been previously harvested for Mt. Gibson or Newhaven reintroduction project and would represent new genetic diversity to the AWC metapopulation.

Table 2. Red-tailed phascogale individuals captured during AWC pre-harvest surveys in 2016–2021. NA indicates a site was not surveyed in a given year.

Remnant	2016	2017	2019	2020	2021
Boyagin	1	1	NA	NA	NA
Dryandra	0	1	NA	1	1
Pingeculling	1	21	12	1	NA
Boundain	4	6	13	1	17
East Yornaning	3	2	0	NA	5
Tutanning	5	4	4	4	NA
Dongolocking	10	7	9	1	12
Jaloran	0	5	12	1	NA
West Ashby	9	13	6	7	3
Quinn's Block	NA	NA	4	1	0
Contine Block	NA	NA	NA	1	2
Montague	NA	NA	NA	NA	7

3.2. Captive Breeding and Translocations to Establish Metapopulation

The ASDP breeding program produced 175 individuals for release in 2020–2021. The Zoos SA program produced 54 individuals for release in 2021–2023. In June 2020, 29 individuals were released in the Newhaven Sanctuary from the ASDP program, 61 were released in November 2020, and 25 were released in April 2020. In November 2021, 60 individuals from ASDP were released to the fenced reserve in Mallee Cliffs National Park. Individuals from the Zoos SA program were subsequently released at Mallee Cliffs NP, with 13 released in March 2022, 20 released in November 2022, and 21 released in March 2023. The Scotia Sanctuary will be founded with individuals from the Zoos SA breeding program. All three sites will benefit from genetic representatives from multiple wild sites while having a different genetic mix of the alleles sampled from the wild.

3.3. Genetic Data

3.3.1. Sequencing and SNP Calling

There were five samples that failed sequencing due to insufficient DNA quantity or quality. After filtering with the 'populations' module, 12,216 SNPs were called from the Stacks pipeline. The sequencing error rate between technical replicates prior to additional filtering averaged 2.2%. SNPs with a reproducibility <90% were removed from analysis, as were SNPs with an MAF < 0.01, to improve the reliability of the SNP data set. After filtering, 4205 SNPs were retained for population genetic analysis.

3.3.2. Population Genetic Analyses

Populations with less than 10 individuals were not included in the analysis (Montague, N = 2; Quinn's Block, N = 1). Observed heterozygosity and allelic richness were highest in the ASDP offspring, followed by the Zoos SA offspring (Table 3). Only one site showed evidence of statistically significant inbreeding (Boundain: F_{IS} = 0.039). The average pairwise mean kinship values within sites were reasonably low (maximum of 0.024 in the Zoos SA offspring).

The PCoA of the ASDP founding sites with the captive offspring showed differentiation between the founding wild sites (Figure 3). Axis 1 explained 8.1% of the variation and was driven by the differentiation between Boundain and Pingeculling. Axis 2 explained 5% of the variation, driven by the Jaloran samples. The ASDP offspring appeared to show a mix of Jaloran, Pingeculling, and some Boundain genetics. The fastSTRUCTURE analysis confirmed that the ASDP offspring are representative of the three wild clusters. When considering K = 3, Boundain is not as well represented in the ASDP offspring. However, when considering K = 2, the combined Boundain–Jaloran cluster is represented well in captivity (Figure 4). For Zoos SA, the captive-born offspring sampled appeared to reflect the genetic structure of the wild-born founders (Figure 3). The fastSTRUCTURE analysis identified K = 1 as the optimum cluster. Overall, genetic differentiation (F_{ST}) between all sites was low (Table 4). Considering all founding populations together with both Zoos SA

and ASDP captive-born offspring, fastSTRUCTURE analysis indicated two optimal clusters (Figure 4).

Table 3. Summary of genetic diversity statistics for ASDP offspring, Zoos SA offspring, and 6 source populations of red-tailed phascogale based on 4205 SNPs. N = sample size, AR = allelic richness, H_O = mean observed heterozygosity, H_E = expected heterozygosity, H_S = standardised heterozygosity (relative to each individual included in the analysis), SE = standard error, F_{IS} = inbreeding coefficient, CI = 95% lower and upper confidence intervals, MK = mean kinship based on estimates of molecular relatedness.

	N	AR (±SE)	H_O (±SE)	H_E (±SE)	H_S (±SE)	F_{IS} (95% CI)	MK (95% CI)
ASDP offspring	18	1.575 (0.006)	0.204 (0.003)	0.202 (0.003)	1.049 (0.014)	−0.009 (−0.051, 0.024)	0.016 (0.012, 0.022)
Zoos SA offspring	40	1.566 (0.005)	0.200 (0.003)	0.200 (0.003)	0.996 (0.030)	−0.001 (−0.022, 0.020)	0.024 (0.019, 0.033)
Boundain	42	1.548 (0.006)	0.188 (0.003)	0.196 (0.003)	0.963 (0.015)	0.039 (0.012, 0.062)	0.017 (0.013, 0.025)
Dongolocking	14	1.545 (0.006)	0.194 (0.003)	0.194 (0.003)	0.990 (0.025)	0.001 (−0.054, 0.041)	0.006 (0.005, 0.009)
Jaloran	13	1.538 (0.006)	0.198 (0.003)	0.194 (0.003)	1.014 (0.050)	−0.019 (−0.132, 0.050)	0.017 (0.014, 0.023)
Pingeculling	15	1.517 (0.006)	0.189 (0.003)	0.188 (0.003)	0.960 (0.029)	−0.007 (−0.072, 0.040)	0.020 (0.018, 0.025)

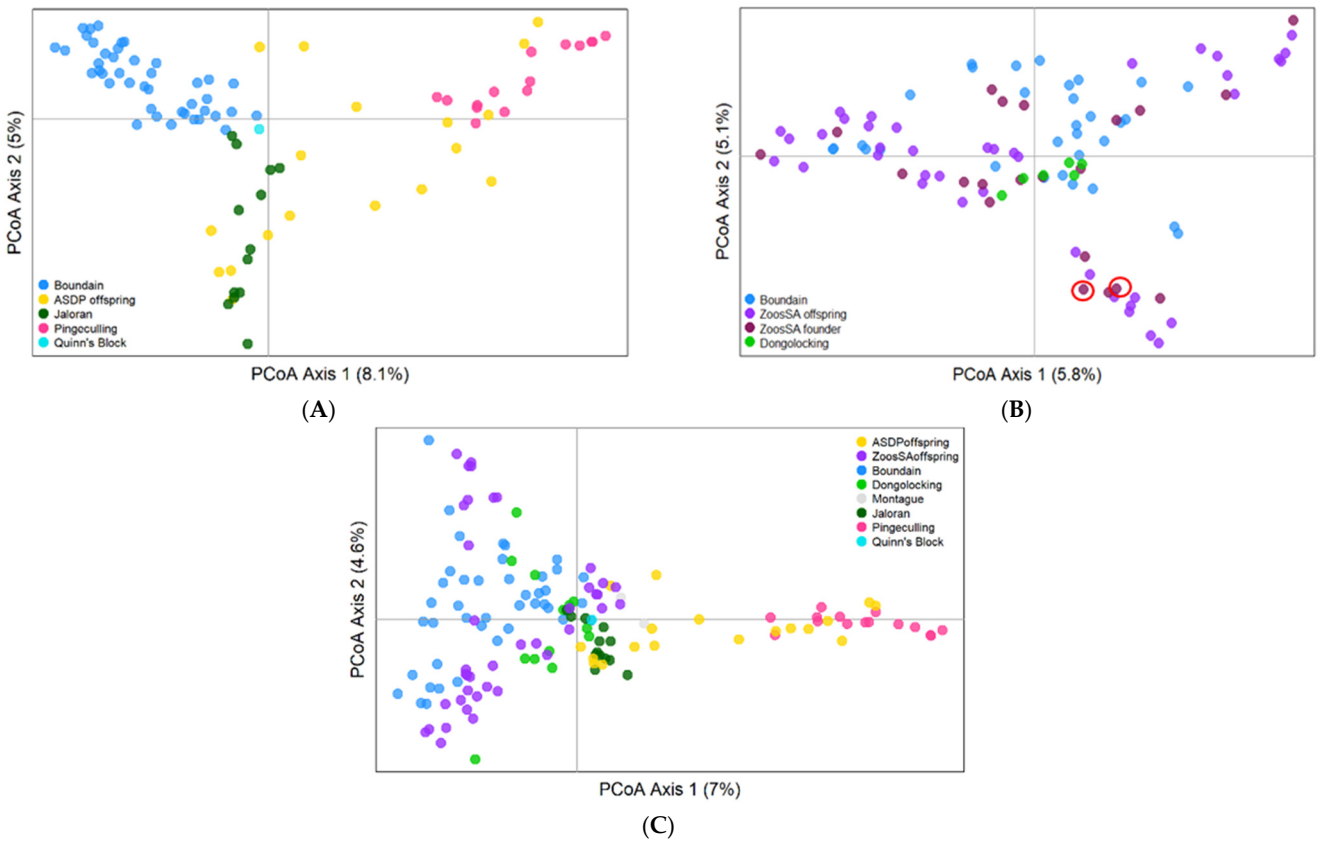

Figure 3. (**A**) PCoA plot of ASDP offspring and the wild source sites used to found the population. Note that the actual founders of the captive population are unknown. (**B**) PCoA plot of the Zoos SA population (both offspring and known founders) and the wild source sites. The two wild Montague samples were both founders of the captive population and are circled in red. (**C**) PCoA of Zoos SA offspring, ASDP offspring, and all wild source sites.

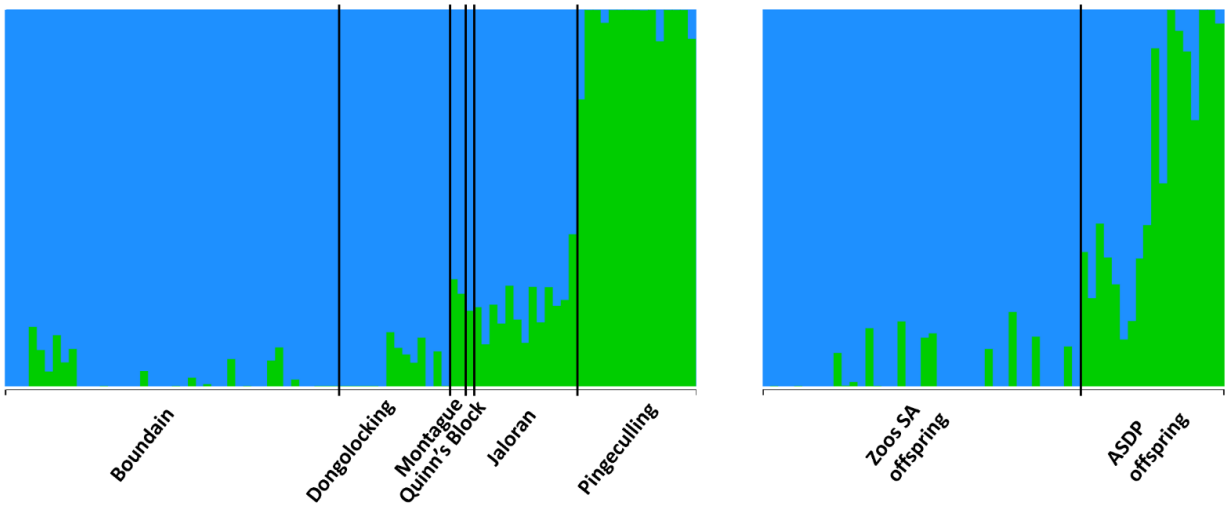

Figure 4. fastStructure plot of K = 2 for all wild source sites and both captive populations of red-tailed phascogales.

Table 4. Population differentiation statistics (F_{ST} values) between two captive and four source populations of red-tailed phascogales with CI = 95% lower and upper confidence intervals below the diagonal and *p*-values above the diagonal.

	ASDP Offspring	Zoos SA Offspring	Boundain	Dongolocking	Jaloran	Pingeculling
ASDP offspring	**NA**	<0.001	<0.001	<0.001	<0.001	<0.001
Zoos SA offspring	0.055 (0.052, 0.059)	**NA**	<0.001	<0.001	<0.001	<0.001
Boundain	0.053 (0.049, 0.057)	0.020 (0.018, 0.022)	**NA**	<0.001	<0.001	<0.001
Dongolocking	0.061 (0.057, 0.066)	0.021 (0.019, 0.024)	0.057 (0.053, 0.062)	**NA**	<0.001	<0.001
Jaloran	0.041 (0.037, 0.044)	0.056 (0.052, 0.059)	0.062 (0.058, 0.066)	0.051 (0.047, 0.056)	**NA**	<0.001
Pingeculling	0.045 (0.042, 0.049)	0.106 (0.100, 0.113)	0.115 (0.109, 0.122)	0.111 (0.105, 0.119)	0.114 (0.108, 0.121)	**NA**

4. Discussion

The application of genetic data to the management of captive breeding programs has been shown to improve outcomes when managing genetic diversity [42]. However, it is often impractical to obtain genotype data in time to use them for decisions relating to breeding pairs when establishing a breeding program. The iterative genetic management of the captive breeding programs established for red-tailed phascogales facilitated a greater range of diversity represented both within the breeding program and subsequently in the translocated populations than could have been achieved without assessing empirical genetic outcomes during the process.

Genetic principles can inform captive management without genetic data, which is often necessary when establishing programs due to the time lag to obtain genomic sequencing results. The practical logistics of setting up breeding programs take priority, and often species need to be paired quickly to encourage positive breeding results. The short life span and breeding cycle of red-tailed phascogales meant that establishing breeding was a priority over waiting for genetic information. Simple principles of pairing individuals from different geographic sites can minimise the potential that individuals are not directly

related, and sites that are more distant geographically are often less genetically similar in species with limited dispersal. The available source sites for red-tailed phascogales were relatively close geographically but fragmented by land clearing. Without genetic data on wild populations, there was uncertainty on assumptions in genetic differences between sites and how that may impact assumptions on relatedness between founders [8]. In fact, the sites all had significant genetic differentiation (as measured by F_{ST}) despite being in relatively close proximity, likely due to relatively recent habitat fragmentation and isolation. While rules of thumb on collecting founders can be informative [43], genetic outcomes in captive breeding can be challenging due to high variability in individual reproductive success [44].

The adaptive genetic management approach of the red-tailed phascogales' breeding-for-release program led to a more genetically representative metapopulation established through reintroductions. Despite collecting founders from four sites to establish the breeding program at ASDP, the genetic signature was dominated by Pingeculling, Jaloran, and a mix between the two. Genomic analysis showed that the Boundain (and Quinn's Block) site was genetically different to Pingeculling and Jaloran but was not well-represented in the ASDP program. This biased representation could be due to the differential breeding success of the males rotated into the female enclosures, combined with the differential breeding success of females in the initial phase of the program when breeding was less successful than in the subsequent breeding season. The group housing of females, and shared use of nest boxes by females housed together, made it difficult to control pairing and offspring identification, especially as preference was made to minimise interference for both welfare and program outcomes.

Conducting the genetic analyses before and after the establishment of the second breeding program facilitated decision making on establishing the second breeding program and allocating offspring to translocation sites to maximise diversity across the metapopulation. An initial aim of establishing the two breeding programs was a breed-for-release program to establish populations at two Australian Wildlife Conservancy (AWC) managed properties, the Newhaven Sanctuary and Mallee Cliffs National Park. The second wild collection targeted the Boundain site, which was shown to have diversity not well represented in the ASDP program, as well as Dongolocking, a site geographically further away from the Pingeculling site that was well-represented in the ASDP program and genetically different from other sites. The second stage of the genetic analysis showed that the Zoos SA program was a good genetic mix of the Boundain and Dongolocking sites and represented a different genetic cluster than the ASDP program. Both breeding programs had higher allelic diversity and heterozygosity than any of the wild source sites, suggesting the use of multiple source sites for both programs led to increased diversity in offspring used to found the translocated sites.

A second goal was to maximise the metapopulation diversity of translocated populations, which was achieved by using different proportions of individuals from each breeding program as founders for each wild site. The Newhaven Sanctuary was founded with 100% ASDP offspring, Mallee Cliffs National Park was founded with ~50/50 ASDP and Zoos SA offspring, and the Scotia Sanctuary will be founded with 100% Zoos SA offspring; thus, all three sites will have different proportions of the two genetic clusters identified represented. A population of red-tailed phascogales had been established previously at another AWC property, Mount Gibson, primarily from a retired research population at the University of Sydney and some additional wild founders. The result is four AWC-managed populations that are genetically diverse, therefore not at immediate risk of inbreeding, but also genetically different to maximise conserving allelic diversity across the populations.

Translocations are becoming more common as a management approach to assist in the recovery of threatened species [2,3]. As translocations and plans for reintroduced populations increase, so does the pressure for source populations to provide high-quality individuals as founders [45]. By managing both captive and translocated populations to maximise diversity within populations [8] while also maintaining diversity between populations, the options for long-term management using captive and translocated sources are increased. Given the fenced reserves have limited carrying capacity, ongoing genetic

management may be necessary to counteract genetic drift over time. At the metapopulation level, the goal will be to balance the objective of minimising inbreeding within populations without homogenising all the populations through frequent assisted gene flow. Maintaining multiple populations that have healthy levels of genetic diversity while representing somewhat different genetic signatures provides long-term genetic management options.

Species that are in need of conservation breeding programs have typically declined significantly and are only remaining in small remnant populations. Wild harvest of these populations can be minimised by careful planning and long-term thinking about how to create populations that are not just for insurance but actively contribute to the recovery of the species [46].

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