Biological Conservation 219 (2018) 1–11

Contents lists available at ScienceDirect

Biological Conservation

journal homepage: www.elsevier.com/locate/biocon

Long-term genetic consequences of mammal reintroductions into an Australian conservation reserve

Lauren C. White[a,b,*], Katherine E. Moseby[c,d,e], Vicki A. Thomson[e], Stephen C. Donnellan[f], Jeremy J. Austin[a]

[a] Australian Centre for Ancient DNA, School of Biological Sciences, University of Adelaide, Adelaide, South Australia 5005, Australia
[b] Max Planck Institute for Evolutionary Anthropology, Department of Primatology, Deutscher Platz 6, Leipzig 04103, Germany
[c] Arid Recovery Ltd, Roxby Downs, South Australia 5725, Australia
[d] University of New South Wales, Sydney, Australia
[e] School of Biological Sciences, University of Adelaide, Adelaide, South Australia 5005, Australia
[f] South Australian Museum, North Terrace, Adelaide, South Australia 5000, Australia

ARTICLE INFO

Keywords:
Arid Recovery
Bilby
Bandicoot
Stick-nest rat
Bettong
Genetic diversity
Translocation
RAD-seq
Admixture
Inbreeding

ABSTRACT

Reintroduction programs aim to restore self-sustaining populations of threatened species to their historic range. However, demographic restoration may not reflect genetic restoration, which is necessary for the long-term persistence of populations. Four threatened Australian mammals, the greater stick-nest rat (*Leporillus conditor*), greater bilby (*Macrotis lagotis*), burrowing bettong (*Bettongia lesueur*) and western barred bandicoot (*Perameles bougainville*), were reintroduced at Arid Recovery Reserve in northern South Australia over the last 18 years. These reintroductions have been deemed successful based on population growth and persistence, however the genetic consequences of the reintroductions are not known. We generated large single nucleotide polymorphism (SNP) datasets for each species currently at Arid Recovery and compared them to samples collected from founders. We found that average genetic diversity in all populations at the Arid Recovery Reserve are close to, or exceeding, the levels measured in the founders. Increased genetic diversity in two species was achieved by admixing slightly diverged and inbred source populations. Our results suggest that genetic diversity in translocated populations can be improved or maintained over relatively long time frames, even in small conservation reserves, and highlight the power of admixture as a tool for conservation management.

1. Introduction

Reintroduction programs aim to establish self-sustaining populations that do not require significant long-term management. Successful reintroductions generally increase a species' population size and geographic range, and restore ecological function to the area from which it was extirpated (Armstrong et al., 2015). Measuring an increase in population growth and size is most often how these reintroduction programs are judged to have succeeded (Ewen et al., 2012; Moseby et al., 2011). However, the ability of a population to persist in the long-term will also be strongly influenced by levels of genetic diversity (Cochran-Biederman et al., 2015, Weeks et al., 2015).

Reintroduced populations are susceptible to loss of genetic diversity due to founder effects, the isolated nature of reintroduction sites, and small population size (Frankham et al., 2010). These circumstances result in unavoidable inbreeding and genetic drift, leading to reduced fitness through the accumulation of deleterious alleles (genetic load), and the increased expression of recessive deleterious traits (inbreeding depression). Additionally, loss of genetic diversity will diminish the adaptive capacity of a population and limit its ability to cope with environmental change (Groombridge et al., 2012).

Thus, most reintroduction programs adopt the preservation of genetic diversity as an explicit goal. Several guidelines can be followed to maximise genetic diversity in reintroduced populations, such as using large numbers of genetically diverse individuals as founders and encouraging rapid population growth after establishment (Jamieson and Lacy, 2012). However, it may not always be possible to follow these guidelines and many other interacting factors, such as the life-history traits and demographic history of a species, may affect genetic diversity in cryptic ways. It is therefore important that genetic monitoring is used in all reintroduction programs to evaluate success and guide management actions to maximise the retention of genetic diversity (Schwartz et al., 2007).

Most studies assessing genetic diversity in reintroduction programs

* Corresponding author at: Max Planck Institute for Evolutionary Anthropology, Department of Primatology, Deutscher Platz 6, Leipzig 04103, Germany.
E-mail address: lauren_white@eva.mpg.de (L.C. White).

https://doi.org/10.1016/j.biocon.2017.12.038
Received 22 September 2017; Received in revised form 19 December 2017; Accepted 24 December 2017
Available online 05 January 2018
0006-3207/

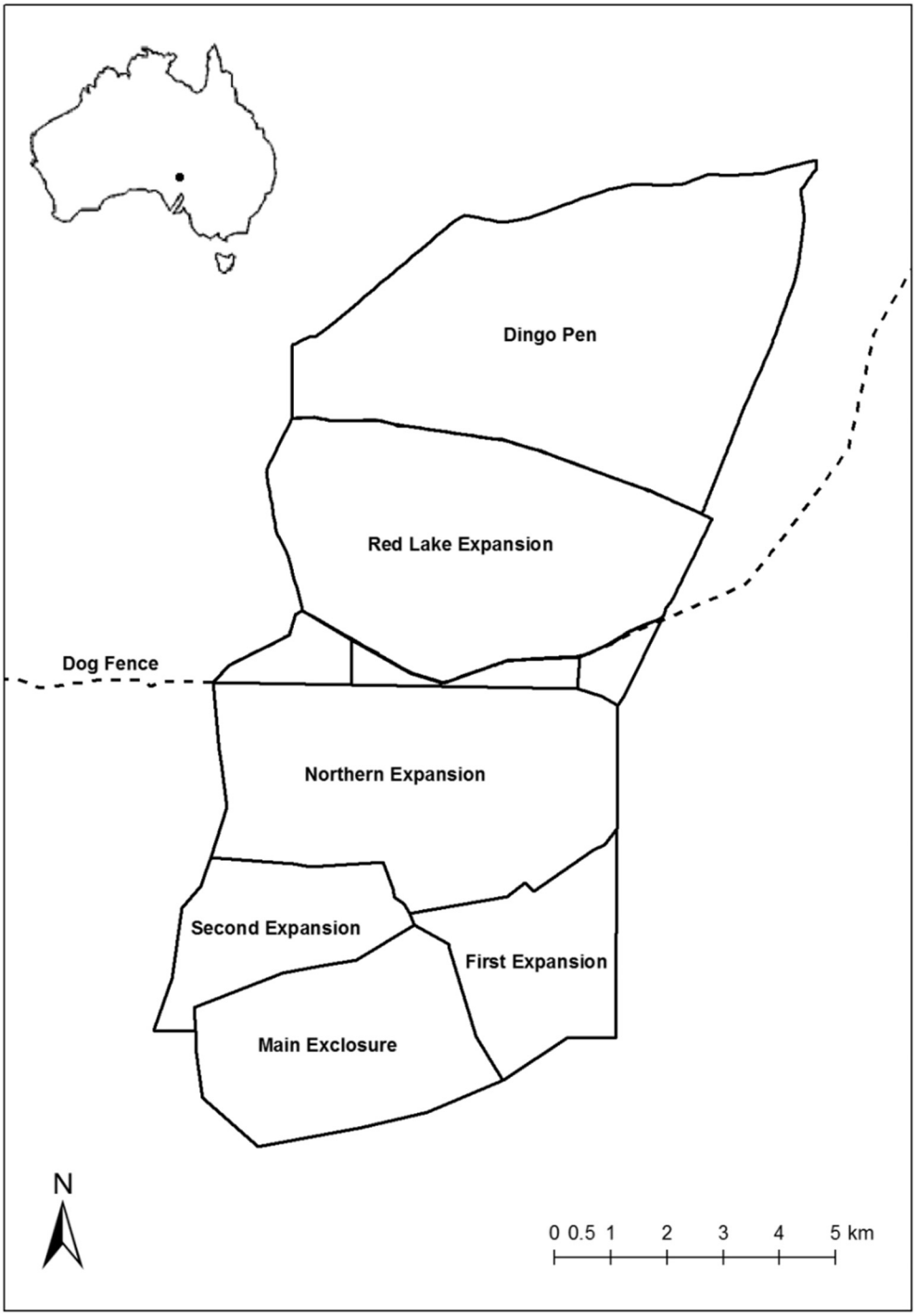

Fig. 1. Location and lay-out of Arid Recovery reserve. Rabbits, cats and foxes have been removed from the four southern paddocks of the Reserve.

have sampled the source and reintroduced populations simultaneously a number of years after release—for example *Gongylomorphus bojerii* (Michaelides et al., 2015) and *Notionmystis cincta* (Brekke et al., 2011)—or sampled just the reintroduced population at multiple time-points—such as *Vulpes velox* (Cullingham and Moehrenschlager, 2013) and *Mustela nigripes* (Cain et al., 2011). In contrast, relatively few studies have explicitly tested changes in genetic diversity from founders to descendants over multiple generations (e.g. Moraes et al., 2017). Such data is crucial for validating and establishing guidelines for maximising genetic diversity in reintroduced populations.

The Arid Recovery Reserve reintroduction program provides a model system in which to compare founder and descendant genetic diversity, as tissue samples were taken from founding individuals at time of release and stored explicitly for later genetic analysis. The reserve is a 123 km^2 fenced exclosure situated 20 km north of Roxby Downs in arid South Australia (Fig. 1). A netting fence surrounds the reserve, and all European rabbits (*Oryctolagus cuniculus*), cats (*Felis catus*), and foxes (*Vulpes vulpes*) have been removed from a 60 km^2 sector at the southern end (Moseby and Read, 2006). Since 1998, this has allowed four species of locally extinct mammals to be reintroduced within the exclosure (Moseby et al., 2011), namely the greater stick-nest rat (GSNR, *Leporillus conditor*), greater bilby (*Macrotis lagotis*), burrowing bettong (*Bettongia lesueur*), and western barred bandicoot (WBB, *Perameles bougainville*). These species were all once widespread across the Australian arid zone, but their geographic ranges have been severely reduced due to competition with grazing stock and rabbits, and

predation from introduced cats and foxes (Burbidge and McKenzie, 1989; Morton, 1990; Newsome, 1971; Richards, 2005).

The reintroductions at Arid Recovery have been deemed successful based on the species' continued survival, population recovery after drought and increased abundance and distribution within the reserve (Moseby et al., 2011). However, the small number of founders ($n = 17 – n = 122$) and fluctuating population size in some species make loss of genetic diversity and inbreeding depression a concern, raising practical questions about the need for additional translocations (i.e. genetic rescue).

Here we compare genetic diversity, using large single nucleotide polymorphism (SNP) datasets, between founders and the descendant populations 18 years after the first reintroductions at Arid Recovery (seven years since the last animal was released). This allowed us to directly measure changes in genetic diversity and accumulation of inbreeding in the descendant populations. We make recommendations regarding the need for genetic rescue at Arid Recovery and, more broadly, comment on reintroduction strategies that can be used to maintain genetic diversity in small, reintroduced populations.

2. Materials and methods

2.1. Population History and Sample Collection:

The reintroduction histories of all four species at Arid Recovery are summarised in Table 1, and detailed descriptions are given in the Supplementary Information. Since release, the bettong population at Arid Recovery has increased rapidly with minimal population fluctuations. The WBB population has also increased without substantial bottlenecks, but at a slower rate than the bettongs (Moseby et al., 2011). Conversely the bilby and GSNR populations have often fluctuated significantly since release in response to seasonal conditions with populations doubling in size and then crashing to < 100 individuals during droughts (Moseby and O'Donnell, 2003; Moseby and Bice, 2004; Moseby et al., 2011).

Population sizes at Arid Recovery at the time of sampling were estimated from track count data for the GSNRs, bilbies and WBBs, and from mark-recapture data for the bettongs (Table 1). As of 2016 there were approximately 500 GSNRs, 500 bilbies, 6000 bettongs, and 1000 WBBs at Arid Recovery (Arid Recovery unpublished data; Moseby, *pers. comm.*).

Founding individuals were DNA sampled as follows: a small (2 mm) ear tissue sample was taken from bettongs and bandicoots, and a 2 mm piece of the tail tip was taken from GSNRs. Samples were not taken from any of the bilby founders, the five WBB founders from Faure Island, and 32 of the GSNR founders originating from Reevesby Island (released in 1998 [$n = 6$] and1999, [$n = 8$]), and Monarto (released in 1998 [$n = 2$] and 2003 [$n = 16$]). Eight WBB ear-clip samples were collected on Faure Island in 2007, and these were used as a proxy for the Faure WBB founders. All founding samples were accessioned after collection in the Australian Biological Tissue Collection (ABTC) at the South Australian Museum, and subsampled for this study.

Post-release DNA samples were obtained during routine monitoring programs or targeted trapping and capture opportunities. WBBs and bettongs were sampled in 2014, while GSNRs and bilbies were sampled in 2016 (Table 1). Trapping at Arid Recovery was conducted under an ethics permit from the South Australian Wildlife Ethics Committee (58–2015). Ear tissue samples were taken using an ear punch or small sharp scissors and stored frozen in 70% ethanol. The numbers of samples collected for different populations and species are summarised in Table 1.

2.2. DNA extraction and ddRAD-seq library preparation

DNA was extracted from tissue samples using a salting out method (Rivero et al., 2006) and the extracts quantified using the Quantus Fluorometer (Promega) as per manufacturer's instructions.

Double-digest Restriction Associated DNA sequencing (ddRAD-seq) libraries were made in batches of 96 including a library blank control following the protocol of Poland et al. (2012) with some modifications. Three hundred nanograms of DNA was digested at 37 °C for 2 h using 8 U of *PstI* (six-base recognition site, CTGCAG) and *HpaII* (four-base recognition site, CCGG) in 20 μL of 1 × CutSmart Buffer (New England Biosciences [NEB]).

Uniquely barcoded adapters (see SI methods and SI Table 1) were then ligated to the DNA in 40 μL consisting of 20 μL of digested DNA, 200 U of T4 ligase, 0.1 ρmol of forward (rare) and 15 ρmol of reverse (common) adapters (SI Fig. 1), and 1 × T4 Buffer. The mixture was incubated at room temperature for 2 h, and then heat killed at 65 °C for 20 min. Ligation products were pooled into 12 pools of eight samples. Pooled libraries were purified using the QIAquick PCR purification kit (Qiagen) and eluted in 120 μL of EB buffer (Qiagen).

Polymerase chain reactions (PCR) to add the full-length Illumina adapters (Poland et al., 2012) were performed in eight replicates per library pool in 30 μL volumes containing 10 μL of purified library, 1 × Hot Start Taq Master Mix (NEB), and 0.66 μM each of the forward and reverse primers (SI Fig. 1). The PCR conditions were: 95 °C for 30 s, 16 cycles of 95 °C for 30 s, 65 °C for 20 s, and 68 °C for 30 s, followed by 68 °C for 5 min, and 25 °C for 1 min. The eight replicates per library were re-pooled and purified as above, eluting in 30 μL of EB buffer (Qiagen). We used a two-step double-SPRI protocol (Lennon et al., 2010) to select for fragments between 100 and 300 bp using a home-made SPRI bead mix (Rohland and Reich, 2012). Libraries were quantified using Tapestation 2200 (Agilent) and pooled at equi-molar concentrations. Pooled libraries were sequenced in 1 × 75 bp (single-end) high output reactions on the Illumina Next-seq at the Australian Genome Research Facility, Adelaide.

Table 1
Reintroduction and genetic sampling history of the four species translocated to Arid Recovery Reserve (AR). Samples were not available from any of the bilby founding individuals, 32 GSNR founding individuals (14 from Reevesby Island and 18 from Monarto) and the Faure Island founding WBB individuals. We sourced eight WBB samples taken from Faure Island in 2007 as proxies for the AR founders, denoted here with an asterisk. Population size at AR was estimated at the time of sampling from track count data (GSNRs, bilbies and WBBs) or mark recapture data (bettongs).

Species	Year of translocation to AR	Number of founders (Male:Female)	Source population (s)	Founders sampled at time of release	Year of sampling at AR	Samples collected at AR	Population size at AR (at time of sampling)
Greater stick-nest rat (GSNR)	1998,1999 & 2003	122 (65:57)	Reevesby Island (98), Monarto (24)	Reevesby:84, Monarto:6	2016	20	500
Greater bilby	2000, 2001 & 2005	37 (21:16)	Monarto (9) Thistle Island (28)	–	2016	16	500
Burrowing bettong	1999 & 2000	30 (11:19)	Heirisson Prong (10) Bernier (20)	All	2014	60	6000
Western barred bandicoot (WBB)	2001 & 2009	17 (4:13)	Bernier Island (12), Faure Island (5)	Bernier:10, Faure:8*	2014	18	1000

2.3. Sequence processing

We used STACKS v1.35 pipeline (Catchen et al., 2013, 2011) to process the sequence data for each species separately, employing parameters recommended by Mastretta-Yanes et al. (2015) to minimise errors and maximise SNP recovery. Raw sequencing reads were demultiplexed, truncated to 65 bp, and filtered for overall quality based on the presence of barcodes using the *process_radtags* module. Samples with fewer than 500,000 reads were excluded from further analysis. RAD loci were identified for each sample using the *ustacks* module, requiring a minimum stack read depth of three (m = 3) and a maximum of two nucleotide mismatches (M = 2) between stacks at a locus. Loci with more than three stacks (mls = 3) and more reads than two standard deviations above the mean were filtered as they may map to multiple points on the genome. A 'deleveraging algorithm' was used to try to resolve over-merged loci. A catalogue of consensus loci among individuals for each species was constructed with the *cstacks* module using the *ustacks* output files. Loci were recognized as homologous across individuals if they mismatched at two or fewer bases (n = 3). Alleles were identified in each individual against this catalogue using the module *sstacks*. The module *populations*, was used to remove potential homologs by filtering out loci with heterozygosity > 0.7 and the resulting SNP datasets were output to a PLINK format file (i.e. ped and map files). Finally, the program PLINK (Purcell et al., 2007) was used to filter out loci with > 25% missing data and minor allele frequencies of < 0.05. Although removing loci with low minor allele frequencies prohibits tracing the loss of rare alleles, we believe this conservative step is necessary to avoid incorporating erroneously called SNPs.

2.4. Quality control

Raw sequences from blank control samples were also run through the STACKS pipeline, matching the *ustacks* output to the consensus catalogue of all four species. Our aim was to remove any potentially erroneous loci in our datasets that were also present in the library blank samples. However, upon inspection, none of the loci found in the blank controls were present in any of the final datasets, having been filtered at previous steps of the pipeline.

A subset of samples from each species was sequenced twice (four GSNRs, five bilbys, 12 bettongs and 10 WBBs) in separate libraries to allow the estimation of error rates. Replicate reads were subsampled to 1 million, 750,000, and 500,000 reads to control for sequencing depth. All subsampled replicates were run through the STACKS pipeline as above, matching the *ustacks* output to the previously constructed consensus catalogue for each species. Allelic error rate was then estimated by counting mismatching alleles at loci for which both replicates had been sequenced.

2.5. Genetic diversity

For each species, samples were grouped by founder/descendant population so that comparisons could be made between each founding group and its descendant population. For each group we calculated observed and expected heterozygosity (H_O, H_E) using the program GENODIVE v2.0b27 Meirmans and Van Tienderen, 2004), and allelic richness corrected for sample size (A_R) using the R package *hierfstat* (Goudet, 2005). Individual heterozygosity and inbreeding coefficients (F) were calculated in PLINK (Purcell et al., 2007). We tested for significant differences in average individual heterozygosity and F between the reintroduced population and their founding groups (where available) using a Wilcoxon rank sum test, corrected for multiple testing.

Wang's pairwise relatedness coefficient (*PR*, Wang, 2002) was estimated for all pairs of individuals within each species using the R package *Related* (Pew et al., 2015). *PR* measures the genetic relatedness of two individuals relative to the average genetic similarity in the total sample (Hardy, 2003). Consequently, negative values may be obtained if two individuals are less related than the average in the reference.

2.6. Temporal differentiation

PCA, pairwise F_{ST}, sNMF and Bayescan analyses were performed to test for differentiation between the founders and descendants. The bilby dataset did not include founder samples and so was excluded from these analyses.

We visualised the variation in our datasets and differentiation between founders and descendants by performing a principal components analysis (PCA) in *adegenet v2.0.1* (Jombart, 2008). PCA is a statistical method for exploring datasets that have a large number of measurements; it reduces the variation in the dataset to a few principal components, which can then be projected onto a graph (Reich et al., 2008).

Genetic distance between founding groups (i.e. founders grouped by source population) and descendants was measured as pairwise F_{ST} in Arlequin v3.5. (Excoffier and Lischer, 2010) using the underlying pairwise distance matrix and 10,000 permutations. Significance values were corrected for multiple tests using the Bonferroni correction (Rice, 1989).

We then used the program sNMF v1.2 to estimate the proportional ancestry in each descendant dataset (Frichot et al., 2014). Similar to the widely-used program STRUCTURE (Pritchard et al., 2000), sNMF estimates the proportion of each individual's genome that originated from a specified number of gene pools (K). Unlike STRUCTURE, sNMF is capable of efficiently analysing large SNP datasets and is more robust to many of the demographic assumptions of Hardy-Weinberg and linkage equilibrium (Frichot et al., 2014). We calculated ancestry proportions in our datasets by running ten replicates of K 1–20 with default parameters and chose the best-supported K as the one with the lowest cross-entropy criterion (CEC), as calculated in sNMF.

We tested for signatures of selection using the F_{ST}-outlier method implemented in Bayescan v2.01 using the default settings (Foll and Gaggiotti, 2008). Bayescan estimates the probability that each locus is subject to selection by teasing apart population-specific and locus-specific components of F-coefficients using a logistic regression. Using a reversible jump Markov chain Monte Carlo (MCMC) algorithm, the posterior probability of a locus being under selection is assessed by testing whether the locus-specific component is necessary to explain the observed pattern of diversity, which infers a departure from neutrality. A threshold value to detect selection was set using a conservative maximum false discovery rate (the expected proportion of false positives) of 0.05.

3. Results

3.1. Sequencing results

We successfully sequenced 95 GSNR, 15 bilby, 71 bettong and 35 WBB samples, (summarised in Table 2 and SI Table 2), generating a large SNP dataset (1752–8703 SNPs) for each species. The WBB samples yielded fewer SNPs (n = 1752) than the other species, despite similar sequencing success and locus discovery, suggesting lower average genetic diversity in this species. This is in agreement with previous studies showing very low genetic diversity in WBBs using microsatellite, mitochondrial (Smith and Hughes, 2008), and MHC (Smith et al., 2010) markers.

The average estimated allelic error rates, calculated between pairs of replicates subsampled to varying depths for each species was 1.2–6.6%, (SI Table 3–6). The error rate did not differ with sequencing depth for any species indicating that our cut-off of 500,000 reads per sample was appropriate.

3.2. Genetic diversity and inbreeding

Observed heterozygosity across all groups (i.e. founders from

Table 2
Summary of sequencing statistics for each founder group and descendant population within each species.

Species	Population	Number of samples successfully sequenced	Average number of reads	Average number of loci	Average depth of coverage	Number of SNPs in final dataset	Average missing data
Greater stick-nest rat	Reevesby Island	72	4,148,368.65	142,615.26	20.66	8703	11.90%
Greater stick-nest rat	Monarto	6	2,545,679.00	110,442.33	16.36	8703	15.80%
Greater stick-nest rat	Arid Recovery	17	4,428,737.94	158,270.65	20.55	8703	6.80%
Greater bilby	Arid Recovery	15	5,597,898.73	97,196.40	38.54	6880	13.23%
Burrowing bettong	Bernier Island	18	2,427,230.28	55,023.33	32.76	3775	10.50%
Burrowing bettong	Heirisson Prong	6	748,519.83	27,520.17	20.45	3775	28.30%
Burrowing bettong	Arid Recovery	47	2,633,766.28	52,221.19	35.50	3775	9.40%
Western barred bandicoot	Bernier Island	9	4,775,200.44	71,154.11	44.71	1752	13.80%
Western barred bandicoot	Faure Island	8	2,480,600.38	69,239.75	26.11	1752	11.70%
Western barred bandicoot	Arid Recovery	18	3,821,004.94	66,350.67	41.68	1752	8.60%

Table 3
Average measures of genetic diversity in founding and descendant populations of mammals released at Arid Recovery, with standard deviation in parentheses. Allelic richness corrected for sample size (A_R), and expected and observed heterozygosity (H_E, H_O).

Species	Population	H_E	H_O	A_R
Greater stick-nest rat	Reevesby Island	0.33 (0.001)	0.31 (0.001)	1.33 (0.14)
Greater stick-nest rat	Monarto	0.33 (0.002)	0.30 (0.003)	1.32 (0.21)
Greater stick-nest rat	Arid Recovery	0.30 (0.002)	0.29 (0.002)	1.30 (0.17)
Greater bilby	Arid Recovery	0.31 (0.002)	0.26 (0.002)	1.28 (0.16)
Burrowing bettong	Bernier Island	0.21 (0.003)	0.20 (0.004)	1.21 (0.21)
Burrowing bettong	Heirisson Prong	0.23 (0.004)	0.18 (0.004)	1.22 (0.26)
Burrowing bettong	Arid Recovery	0.34 (0.002)	0.31 (0.002)	1.34 (0.13)
Western barred bandicoot	Bernier Island	0.15 (0.002)	0.14 (0.002)	1.15 (0.19)
Western barred bandicoot	Faure Island	0.13 (0.005)	0.15 (0.006)	1.13 (0.21)
Western barred bandicoot	Arid Recovery	0.24 (0.003)	0.21 (0.003)	1.24 (1.33)

different sites and descendants) ranged from 0.14 to 0.31 and was lower than expected heterozygosity under Hardy-Weinberg equilibrium (HWE) for all populations except for the Faure Island WBBs (Table 3). Allelic richness ranged from 1.13 (Faure Island founder WBBs) to 1.34 (Arid Recovery descendant bettongs). The WBBs had the lowest genetic diversity of the four species, again consistent with previous studies (Smith and Hughes, 2008; Smith et al., 2010).

The bettongs and WBBs at Arid Recovery had higher diversity across all measures, than either of their founding groups. On the other hand, the Arid Recovery GSNR population had slightly lower diversity across all measures than their founders. Although we could not do similar comparisons with the bilby dataset, as founding samples were not available, we note that their diversity measures are similar to the other species at Arid Recovery.

We further explored genetic diversity by calculating individual heterozygosity (Fig. 2). Average individual heterozygosity was significantly higher in the Arid Recovery bettongs compared to its two founding populations ($p < 0.05$), while all other comparisons between populations or groups were non-significant ($p > 0.05$). The distribution of individual heterozygosity within groups of all species demonstrates how genetic diversity is relatively even across individuals within each population, except for within the Arid Recovery WBBs. In this group, five individuals are much more heterozygous than all other samples. Most individuals in the WBB population have lower heterozygosity than the founding group, but the average has been driven up by the five outliers.

The Arid Recovery bettong and WBB populations' average inbreeding were lower than either of their founding groups (Fig. 3). However, only the bettong population had significantly different average inbreeding compared to their founders ($p < 0.05$). The WBB inbreeding was highly variable, with most sampled individuals having higher coefficients than the founders. The five Arid Recovery WBB individuals with high heterozygosity, and therefore, much lower inbreeding coefficients than the rest of the WBB group again drove this pattern. The Arid Recovery GSNR population had slightly higher (although non-significantly, $p > 0.05$) average inbreeding than either of their founding groups, and the Arid Recovery bilby population had comparable average inbreeding to the Arid Recovery GSNR and bettong populations.

Average pairwise relatedness (*PR)* between individuals was higher within the Arid Recovery GSNR population than in either of its founding groups (Fig. 4). Conversely, average *PR* was lower in the bettong and WBB Arid Recovery populations compared to their founding groups (Fig. 4). However, the *PR* in the WBBs was again quite varied, and lowest between the same five individuals that also had lower inbreeding and higher heterozygosity. The *PR* measured in the bettong and WBB populations also show that the two founding groups for each species (Bernier Island and Heirisson Prong in bettongs, and Bernier Island and Faure Island for the WBBs), were highly unrelated to each other and that the WBB Arid Recovery population was more related to its Bernier Island founding group than the Faure Island founding group, excepting the five outlier individuals, which were equally related to both founding groups. *PR* within the Arid Recovery bilby population was varied, but generally low.

3.3. Arid recovery differentiation from founding groups

The results of principle component analysis for the GSNR, bettong and WBB datasets are shown in Fig. 5. The GSNR Arid Recovery population is identifiable as a cluster separate from both founding groups of Monarto and Reevesby Island individuals, although the total amount of variation explained by the first two principle components is low (2.24–3.4%). The Arid Recovery bettong population clusters as a group intermediate between its two founding groups, Bernier Island and Heirisson Prong. Finally, the Arid Recovery WBB samples cluster with its Bernier Island founding group separate to the Faure Island proxy founders. The five WBB individuals with lower inbreeding and higher heterozygosity are the most intermediate between the rest of the Arid Recovery/Bernier Island group and the Faure Island cluster.

Pairwise F_{ST} values for each species are shown in Table 4 and are in general agreement to the PCA results. F_{ST} values between GSNR groups were significantly different from zero between Arid Recovery and the

Fig. 2. Individual observed heterozygosity calculated for each sampled individual of greater stick-nest rat (GSNR), greater bilby, burrowing bettong and western barred bandicoot (WBB). Each vertical bar represents an individual, and is coloured by population. Population names have been shortened: A.R —Arid Recovery; R.I. — Reevesby Island; Mo. — Monarto; H.P. — Heirisson Prong; B.I. — Bernier Island; F.I. — Faure Island.

founding groups, but not between the Monarto and Reevesby Island animals. All pairwise F_{ST} values were significantly different from zero between all groups of bettongs, being highest between the two founding groups (Heirisson Prong and Bernier Island). Within the WBB dataset, pairwise F_{ST} was significantly different from zero between Arid Recovery and the Faure Island group, and between the two founding groups (Faure Island and Bernier Island), but not between Bernier Island and Arid Recovery.

The sNMF analysis inferred that the most likely number of ancestral gene pools was two for the GSNR and bettong datasets, and three for the WBB dataset (SI Fig. 2). Results of the ancestry estimates are shown in Fig. 6. The GSNR plot shows most individuals in this dataset are a mixture of two genepools, with Reevesby Island dominated by one (average of 80% 'blue' in the plot) and Arid Recovery dominated by the other (average of 85% 'red' in the plot). The bettong sNMF plot shows that the Arid Recovery population is a mixture of the Bernier Island (mainly all blue) and Heirisson Prong (mainly all red) founders with an average of 71% Heirisson Prong and 29% Bernier Island ancestry.

The WBB sNMF plot shows that most Arid Recovery individuals share their entire ancestry with the Bernier Island founders. However, seven individuals are estimated (under K = 3) to have ancestry from a third source (shown in orange on the plot). When we plot the ancestry estimates for the WBB dataset under K = 2 (as the known number of sources, Fig. 6) we can see that those seven individuals are those with admixture from the Faure Island population. We also note that the five individuals with the most Faure Island ancestry correspond to the individuals that were found to be the least inbred and most heterozygous.

Bayescan analysis identified six loci under putative selection in the GSNR dataset, but none in the bettong or WBB datasets (SI Fig. 3). These six loci represents 0.07% of the total GSNR dataset and had FST values of >0.19 compared to an average of 0.05 across all loci.

4. Discussion

Despite relatively small founding populations, but perhaps consistent with modest-to-large population growth in all four species over an ~18-year period, our results show that average genetic diversity in all populations of reintroduced mammals at Arid Recovery reserve are close to, or exceeding, the levels measured in their founding groups. We detect only a small reduction in genetic diversity and small increase in inbreeding since release in the GSNR population, while the bettong and WBB populations are, on average, more diverse and less inbred than their founding groups. These results are driven by the mixing of two diverged and individually inbred source populations, which has had a large positive impact on the genetic diversity of the descendant Arid Recovery populations. Our study suggests that additional translocations to Arid Recovery may not be necessary at this time, and highlights the power of admixture, even from small isolated populations, as a tool for conservation management to maximise genetic diversity in threatened taxa via genetic rescue.

GSNRs at Arid Recovery have retained 94–98% of genetic diversity (depending on the measure used) and show no significant increase in inbreeding compared to their founding groups. These results indicate that most of the genetic diversity captured in the founding individuals from Monarto and Reevesby Island has been retained in the Arid Recovery populations, possibly because of the larger-than-average number of founders released (n = 122).

However, we do detect a small amount of differentiation between the GSNR Arid Recovery population and their founding groups, indicated by the small, but significant, pairwise F_{ST} values, and both the

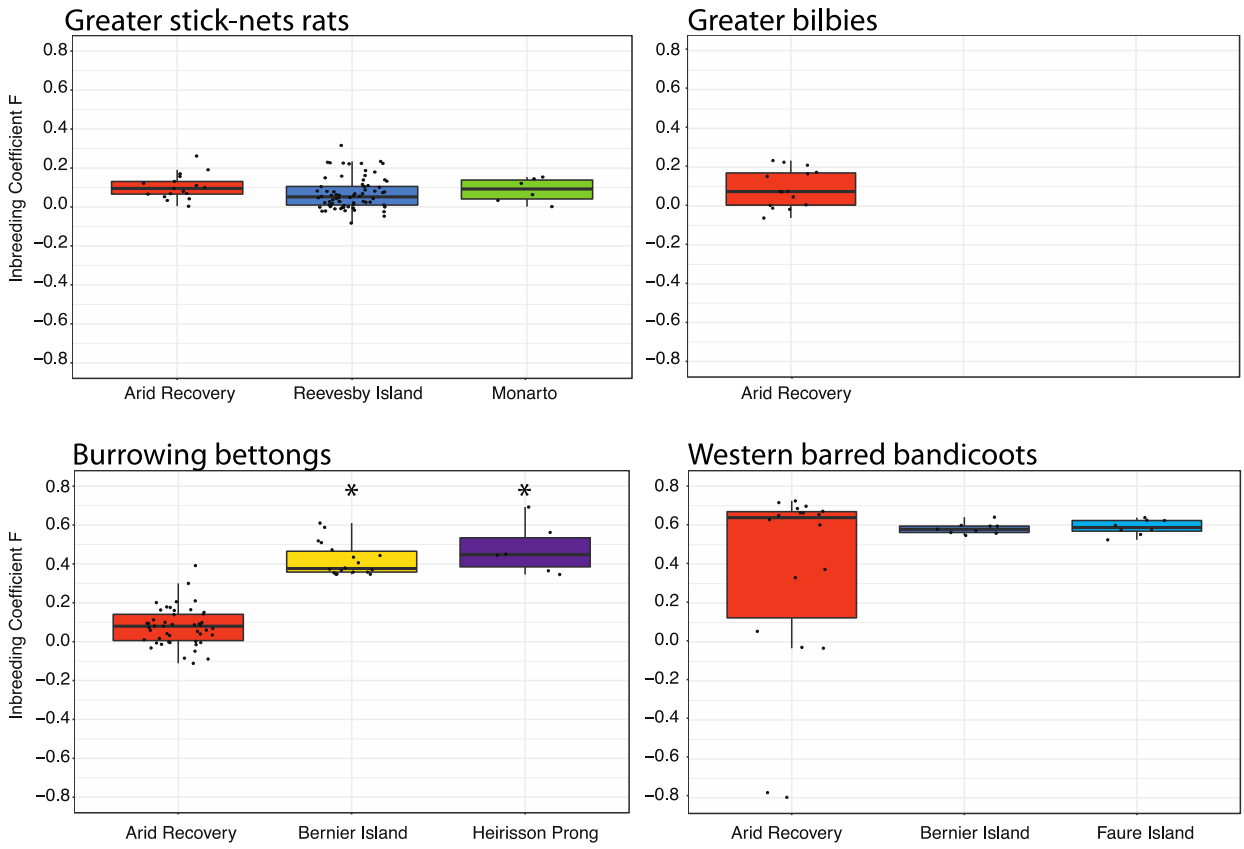

Fig. 3. Individual inbreeding coefficients per population for founding groups (where available) and current Arid Recovery populations of greater stick-nest rats (GSNR), greater bilbies, burrowing bettongs and western barred bandicoots (WBB). Dots represent individual values. Middle horizontal lines represent the median, the boxes are bound by the 25th and 75th quartiles and vertical lines show the minimum and maximum range of values excluding outliers. Founding groups that had significantly different average inbreeding coefficients from their descendant Arid Recovery populations are denoted with an asterisk.

sNMF analysis and PCA plot. This differentiation could be due to selection. For example, unlike the other populations of reintroduced species, the Arid Recovery GSNR population experiences high mortality due to heat stress during summer, which may be acting as a selective pressure in this population (Moseby, *pers. comm*). This hypothesis is partially supported by our Bayescan analysis, which detected six loci under putative selection in the GSNR dataset. However, F_{ST} outliers can also result from demographic effects, such as wave-edge surfing in recently bottlenecked populations (Hofer et al., 2009; Klopfstein et al., 2006). Given the probable small effective population size in the Arid Recovery population that would limit natural selection (Frankham et al., 2010), genetic drift is a more likely explanation for the differentiation seen in the GSNRs here. Further field experiments comparing fitness of locally sourced and translocated animals in the Arid Recovery environment could be used to test the hypothesis of local adaptation in the Arid Recovery population. Such research is crucial to understanding how drift and selection can be differentiated and ultimately how either case should be treated in translocated populations, particularly when animals are moved between climatic zones.

The bettong and WBB populations have increased average genetic diversity compared to their founding groups. Allelic richness has increased in both populations by $>7\%$ and measures of heterozygosity have increased between 40% and 80%. We found that in both species these results were entirely driven by admixture between two diverged sources.

Within the Arid Recovery bettong population, ancestry proportions were relatively similar across individuals, likely reflecting the fact that the two groups of founding individuals (from Bernier Island and Heirisson Prong) were released within a year of each other and have had 16 years to interbreed. It is interesting that, on average, the majority of ancestry (as shown in the sNMF analysis) in Arid Recovery bettongs was from the Heirisson Prong founders, despite only 10 individuals being released from this source compared to 20 from Bernier Island. This may be due to the additional year that the Heirisson Prong founders had to acclimatize to the new habitat before the Bernier Island founders were released, potentially giving the first group an advantage over the second. Although, this pattern could also be driven by stochastic drift.

Within the WBB Arid Recovery population, the admixture is less evenly distributed than in the bettong population, likely because of the smaller number of individuals translocated from the second source, and the shorter time since the second release. Only five individuals were translocated from Faure Island in 2009 (eight years after the first release from Bernier Island), but their genetic impact on the population is clear. Individuals without Faure Island admixture were slightly more inbred and less genetically diverse than the founding groups, while the individuals with admixture had much lower inbreeding and much higher heterozygosity than any other sampled individual. The five outlier individuals had roughly half of their ancestry, as estimated by sNMF analysis, originating from Faure Island which indicates they may be F1 hybrids. The Faure Island WBBs released into Arid Recovery were first contained within a pen and allowed to breed with each other before being released into the wider reserve. Given that WBBs live for three to five years, sampling of F1 hybrids is possible. We expect this admixture in the WBBs to spread throughout the population in subsequent generations. However, to ensure the introgressed genetic diversity is not lost through stochastic processes, the genetic composition of the WBB population should be retested in a biologically relevant time-frame (for example 5–10 generations).

The pattern of admixture in the WBBs compared to that observed in

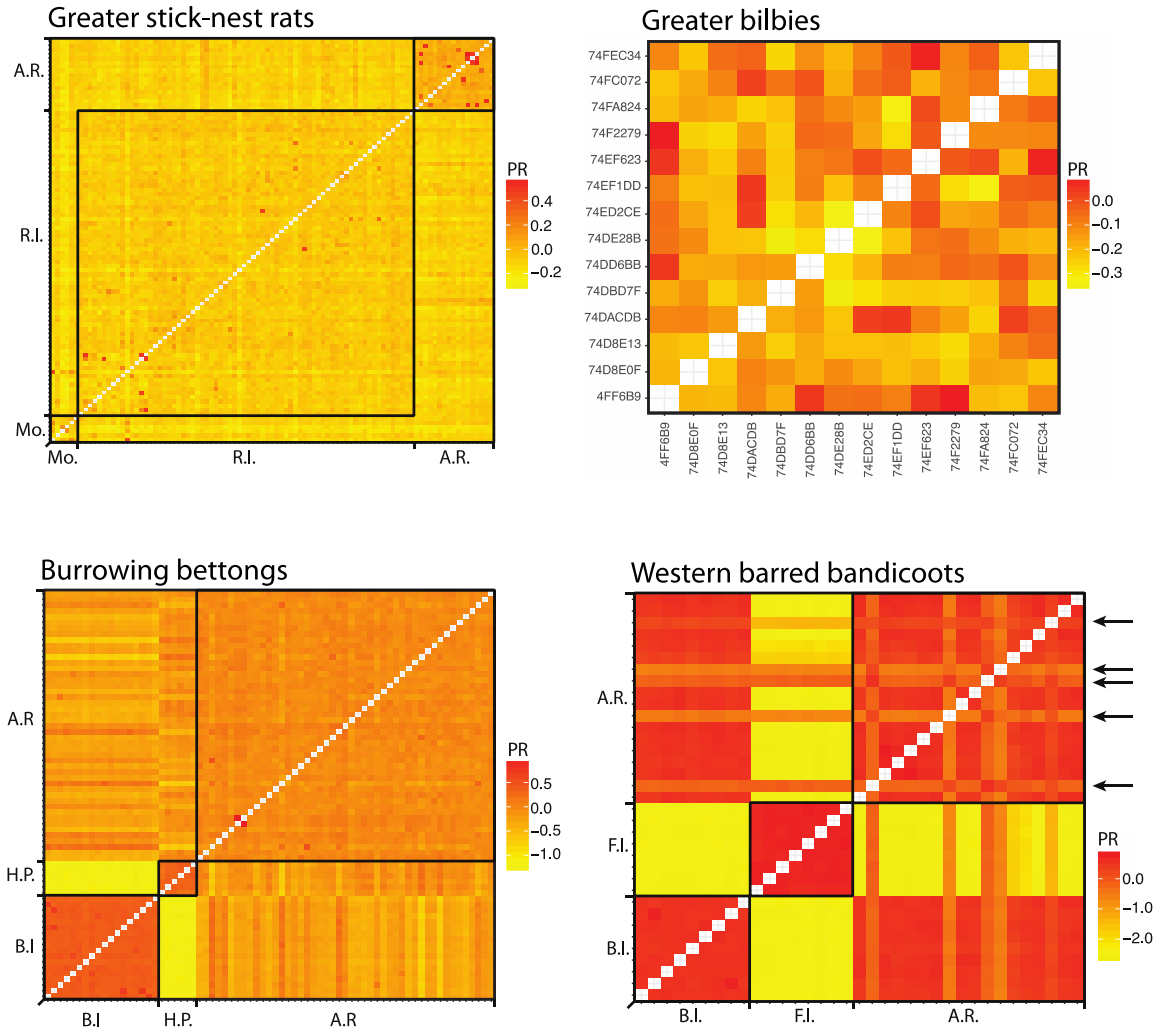

Fig. 4. Heat map of pairwise relatedness (*PR*) calculated between each sampled individual within each species. Within population comparisons are bounded by black squares. Arrows on the WBB heat map highlight the five individuals with lower levels of inbreeding and average pairwise relatedness than the rest of the WBB Arid Recovery samples. Population names are shortened due to space requirements: A.R — Arid Recovery; R.I. — Reevesby Island; Mo. — Monarto; H.P. — Heirisson Prong; B.I. — Bernier Island; F.I. — Faure Island. Bilby PR is labelled by sample as founding individuals were not sampled.

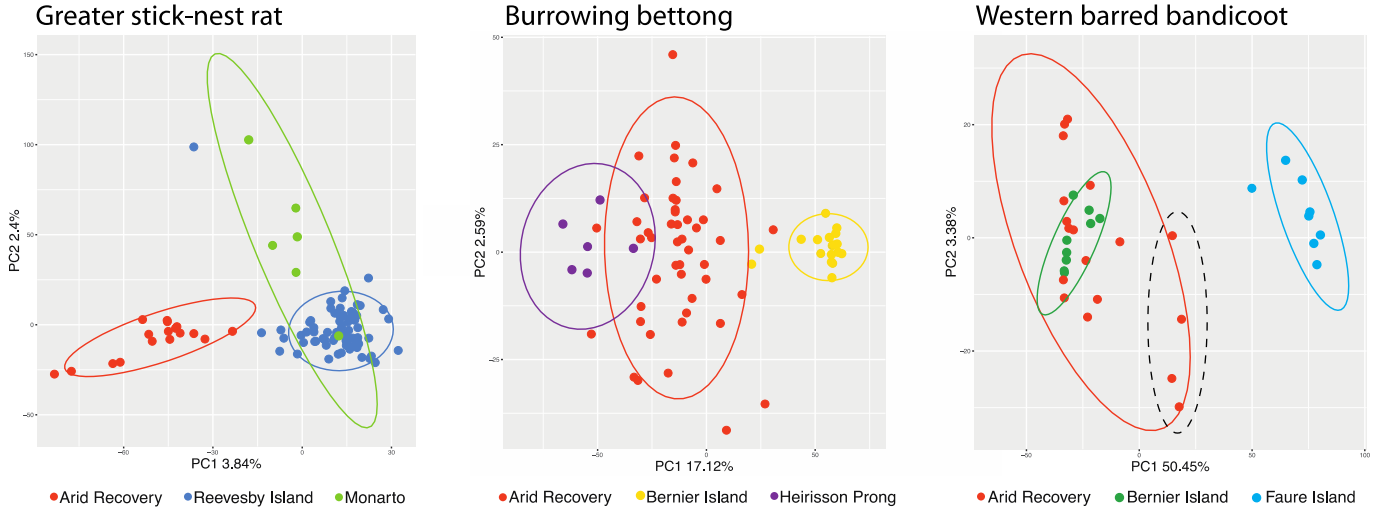

Fig. 5. Relationships among founding groups and the descendant Arid Recovery populations of greater stick-nest rats (GSNR), burrowing bettongs and western barred bandicoots (WBB) based on principle coordinate analysis for principle components 1 and 2. Each dot represents an individual coloured by population. Solid ellipses represent the centre and 95% confidence interval of the points in each population. The dotted ellipse encompasses the five outlier WBB samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
Pairwise F_{ST} values calculated between the founding groups and descendant Arid Recovery populations for the greater stick-nest rats, burrowing bettongs and western barred bandicoots. Significant values (after Bonferroni correction) are highlighted in bold.

Greater stick-nest rats (GSNR)			
	Arid Recovery	Reevesby Island	Monarto
Arid Recovery			
Reevesby Island	**0.04352**		
Monarto	**0.05930**	0.02845	
Burrowing bettongs			
	Arid Recovery	Bernier Island	Heirisson Prong
Arid Recovery			
Bernier Island	**0.19133**		
Heirisson Prong	**0.11992**	**0.53907**	
Western barred bandicoots (WBB)			
	Arid Recovery	Bernier Island	Faure Island
Arid Recovery			
Bernier Island	0.03933		
Faure Island	**0.67165**	**0.8124**	

the bettongs suggests that, where possible, translocation programs should aim to mix a similar number of individuals from different genetic stock simultaneously and early on in the establishment of reintroduced populations to maximise the benefits of admixture on genetic diversity.

The bilby population at Arid Recovery had similar levels of inbreeding and genetic diversity to the GSNR and bettong populations within the reserve. We were, however, unable to assess how much inbreeding had accumulated or how much genetic diversity has been retained since release as samples from the bilby founders were not available. We emphasize the importance of collecting samples from founders during reintroduction programs for use in later genetic assessments, even when individuals are sourced from captive breeding facilities with studbooks. Genotyping samples from other extant populations of bilbies across Australia would improve our inference about how resilient this population is to genetic deterioration. Moritz et al. (1997) examined genetic diversity across the wild bilby range using mitochondrial DNA and microsatellites. Repeating this analysis using SNP data would permit direct comparison with our dataset and allow recommendations on the need for additional translocations to be made.

Given that our results show Arid Recovery Reserve has been successful in maintaining or even increasing the genetic diversity in the species reintroduced there, we suggest additional reintroductions may not be necessary at this time. However, we note that our datasets did not allow us to detect the true impact of founder effects on the Arid Recovery populations. A founder effect is the reduction of genetic diversity in a new population compared to its source resulting from non-representative founding individuals (i.e. when not all genetic diversity present in a source population is 'captured' in the founding individuals; Frankham et al., 2010). We would expect this effect to be exacerbated when serial founder events occur (i.e. when the founding source is itself a reintroduced or captive population), as is the case for some of the Arid Recovery species. Further sampling at source, the original source populations (in the case of serial founding events), and other remnant populations of each species should be prioritised to determine whether genetic diversity can be further increased in the Arid Recovery populations.

A further area of research that we were unable to address here, but that is critical to improving species reintroductions, is the impact of mating strategies, sex ratio and sex-specific reproductive skew on the maintenance of genetic diversity. Our current understanding of the species at Arid Recovery suggests that their mating strategies are similar (eg.polygamous and probably polygynous; the females have tight home ranges and the males' home ranges overlap with several females [Moseby, *pers. comm.*]), precluding comparisons, and, unfortunately, we do not know the level of reproductive-skew (sex biased or otherwise) across founding animals. Future work could address the later by tracking haplotypes of sex-specific loci (Y-chromosome or mitochondrial DNA) from the founders to the descendant population. Alternatively, the impact of these processes, including mating strategy, on genetic diversity in reintroduced populations could be studied using in-silico simulations (eg. Fiumera et al., 2004).

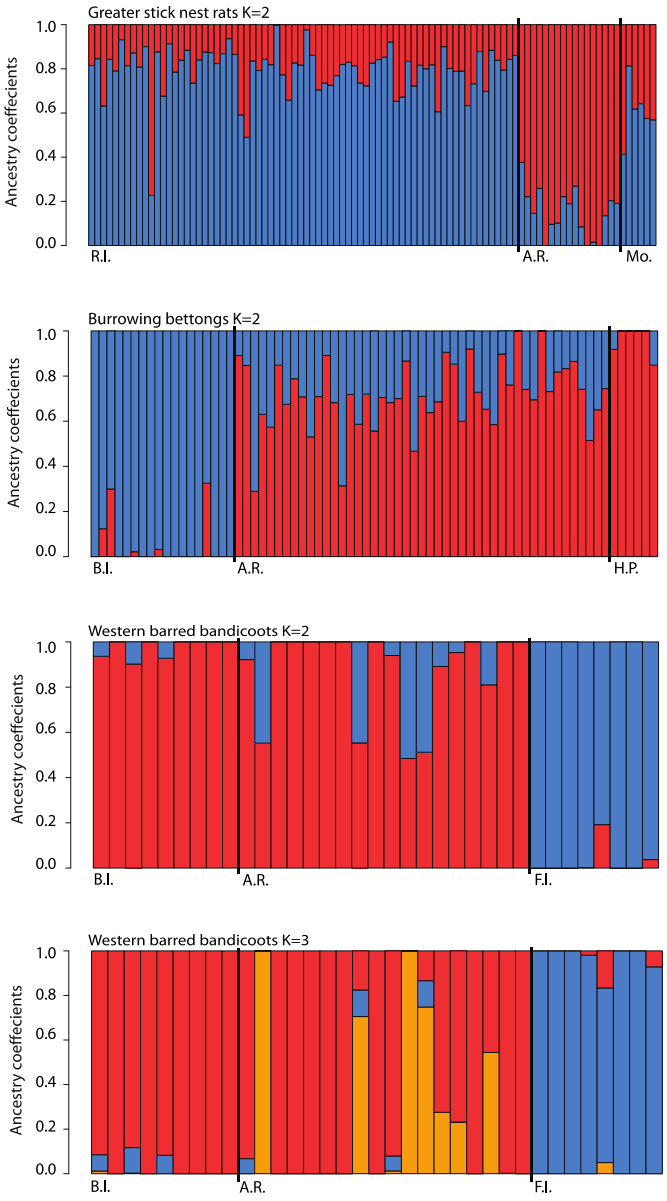

Fig. 6. Genetic ancestry in individuals from Arid Recovery and their founding groups estimated using sNMF. Each vertical bar represents an individual. Population names are shortened due to space requirements: A.R — Arid Recovery; R.I. — Reevesby Island; Mo. — Monarto; H.P. — Heirisson Prong; B.I. — Bernier Island; F.I. — Faure Island.

4.1. Admixture as a conservation tool

Our results highlight the positive impact that admixture has had on genetic diversity in two of the reintroduced mammal populations at Arid Recovery. The impact of admixture and gene flow on genetic diversity is well established. Wright (1931) and Franklin (1980) estimated that just one migrant per generation would be enough to prevent population differentiation, drift and loss of adaptive potential (although more recent work suggests 1–10 migrants per generation may be necessary to stop loss of diversity in wild populations; Mills and Allendorf,

1996). Admixture of diverged populations was found to substantially increase the genetic diversity in reintroduced populations of the peregrine falcon (*Falco peregrinus*; Jacobsen et al., 2008) and Alpine ibex (*Capra ibex*; Biebach and Keller, 2012), even when divergence between the source populations was low. Furthermore, genetic rescue (i.e. deliberate introduction of individuals from other populations to restore genetic diversity and fitness) is an effective strategy to increase the reproductive health of small, inbred populations (Heber et al., 2013; Hedrick and Fredrickson, 2010; Madsen et al., 2004; Weeks et al., 2015).

Despite the evident advantages, admixture has been underutilized as a conservation tool due to concerns about outbreeding depression and the need to conserve locally adapted variation within subpopulations (Frankham, 2015; Weeks et al., 2016, 2011). However, outbreeding depression is unlikely when mixing animals from populations that share similar environments, have the same karyotype, have previously exchange genes and/or have long generation times (Frankham et al., 2010). Furthermore, Weeks et al. (2016) argue that many populations previously perceived as genetically 'unique' and potentially locally adapted using neutral genetic markers, are often more likely to have differentiated through random genetic drift and are therefore the populations most likely to be in need of genetic restoration.

The source populations of the WBBs and bettongs at Arid Recovery are from similar environments, all originating from islands in Shark Bay, Western Australia, and are therefore unlikely to have different local adaptations. Additionally, a previous study found only minor mitochondrial haplotype divergence between the two WBB remnant populations (Smith and Hughes, 2008). Hence, the admixture at Arid Recovery is unlikely to have resulted in outbreeding depression. Rather, the bettong population at Arid Recovery, which was admixed from the outset of the reintroduction program, has seen the most significant population growth of all the reintroduced species at the reserve, suggesting a possible fitness advantage in the admixed animals. Further experiments examining the fitness levels of inbred compared to outbred/admixed bettongs is needed to test this hypothesis. Regardless of whether this admixture confers any fitness advantages in the Arid Recovery populations, mixing of the diverged source populations will contribute to the preservation of adaptive potential in these species.

5. Conclusion

Our high-resolution datasets have revealed the success of the Arid Recovery reintroduction programs in maintaining and maximising genetic diversity of the threatened mammal species released there. Our results suggest that additional translocations to Arid Recovery may be unnecessary at this time, and highlight the clear benefit to reintroduction programs of admixing slightly diverged populations to maximise genetic diversity and adaptive potential in threatened taxa. Comparison of the two admixture strategies employed in the bettong and WBB populations at Arid Recovery show future translocation programs that plan to mix different genetic stocks should aim to release equal numbers of animals from both sources simultaneously, early in the reintroduction program. This will promote balanced admixture of both sources in the descendant population.

Ultimately, we have demonstrated the benefits of genetic monitoring in reintroduction programs and advocate for it's continued use at Arid Recovery and in other reintroduction programs in the future.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocon.2017.12.038.

Data availability

All de-multiplexed raw sequencing data are available from NCBI's short read archive (Accession number: PRJNA389954).

Acknowledgments

Arid Recovery is a conservation initiative supported by BHP Billiton, The University of Adelaide, The South Australian Department for Environment and Natural Resources and the local community. The Nature Foundation of South Australia generously provided funding for this project. We thank the Australia Wildlife Conservancy for providing western barred bandicoot samples from Faure Island. We are indebted to the many Arid Recovery staff and volunteers who assisted with this project including John Read, Jackie Bice, Andrew Freeman, Katherine Tuft, Rebecca West, Reece Pedler, and Kimberly Solly.

Role of the funding source

This work was funded by the Nature Foundation of South Australia, which did not contribute to the design, interpretation or the writing of this study.

References

Armstrong, D., Hayward, M., Moro, D., Seddon, P., 2015. Advances in Reintroduction Biology of Australian and New Zealand Fauna. CSIRO Publishing, Melbourne.
Biebach, I., Keller, L.F., 2012. Genetic variation depends more on admixture than number of founders in reintroduced alpine ibex populations. Biol. Conserv. 147, 197–203.
Brekke, P., Bennett, P.M., Santure, A.W., Ewen, J.G., 2011. High genetic diversity in the remnant island population of hihi and the genetic consequences of re-introduction. Mol. Ecol. 20, 29–45.
Burbidge, A.A., McKenzie, N.L., 1989. Patterns in the modern decline of western Australia's vertebrate fauna: causes and conservation implications. Biol. Conserv. 50, 143–198 Australian Developments in Conservation Evaluation.
Cain, C.M., Livieri, T.M., Swansom, B.J., 2011. Genetic evaluation of a reintroduced population of black-footed ferrets (*Mustela nigripes*). J. Mammal. 92, 751–759.
Catchen, J.M., Amores, A., Hohenlohe, P., Cresko, W., Postlethwait, J.H., 2011. Stacks: building and genotyping loci de novo from short-read sequences. G3 Genes Genom Genet 1, 171–182.
Catchen, J., Hohenlohe, P.A., Bassham, S., Amores, A., Cresko, W.A., 2013. Stacks: an analysis tool set for population genomics. Mol. Ecol. 22, 3124–3140.
Cochran-Biederman, J., Wyman, K.E., French, W.E., Loppnow, G.L., 2015. Identifying correlates of success and failure of native freshwater fish reintroductions. Conserv. Biol. 29, 175–186.
Cullingham, C.I., Moehrenschlager, A., 2013. Temporal analysis of genetic structure to assess population dyamics of reintroduced swift foxes. Conserv. Biol. 27, 1389–1398.
Ewen, J.G., Armstrong, D., Parker, K.A., Seddon, P.J., 2012. Reintroduction Biology: Integrating Science and Management. Wiley-Blackwell, Oxford.
Excoffier, L., Lischer, H., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Resour. 10, 564–567.
Fiumera, A.C., Porter, B.A., Looney, G., Asmussen, M.A., Avise, J.C., 2004. Maximizing offspring production while maintaining genetic diversity in supplemental breeding programs of highly fecund managed species. Conserv. Biol. 18, 94–101.
Foll, M., Gaggiotti, O., 2008. A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a bayesian perspective. Genetics 180, 977–993.
Frankham, R., 2015. Genetic rescue of small inbred populations: meta-analysis reveals large and consistent benefits of gene flow. Mol. Ecol. 24, 2610–2618.
Frankham, R., Ballou, J.D., Briscoe, D.A., 2010. Introduction to Conservation Genetics, 2nd ed. Cambridge University Press, New York.
Franklin, I.R., 1980. Evolutionary change in small populations. In: Conservation Biology: An Evolutionary-Ecological Perspective. Sinauer Associates.
Frichot, E., Mathieu, F., Trouillon, T., Bouchard, G., François, O., 2014. Fast and efficient estimation of individual ancestry coefficients. Genetics 196, 973–983.
Goudet, J., 2005. Hierfstat, a package for r to compute and test hierarchical F-statistics. Mol. Ecol. Notes 5, 184–186.
Groombridge, J., Raisin, C., Bristol, R., Richardson, D.S., 2012. Genetic consequences of reintroductions and insights from population history. In: Reintroduction Biology: Integrating Science and Management. Wiley-Blackwell, Oxford.
Hardy, O.J., 2003. Estimation of pairwise relatedness between individuals and characterization of isolation-by-distance processes using dominant genetic markers. Mol. Ecol. 12, 1577–1588.
Heber, S., Varsani, A., Kuhn, S., Girg, A., Kempenaers, B., Briskie, J., 2013. The genetic rescue of two bottlenecked South Island robin populations using translocations of inbred donors. Proc. R. Soc. Lond. B Biol. Sci. 280, 20122228.
Hedrick, P.W., Fredrickson, R., 2010. Genetic rescue guidelines with examples from Mexican wolves and Florida panthers. Conserv. Genet. 11, 615–626.
Hofer, T., Ray, N., Wegmann, D., Excoffier, L., 2009. Large allele frequency differences between human continental groups are more likely to have occurred by drift during range expansions than by selection. Ann. Hum. Genet. 73, 95–108.
Jacobsen, F., Nesje, M., Bachmann, L., Lifjeld, J.T., 2008. Significant genetic admixture after reintroduction of peregrine falcon (*Falco peregrinus*) in Southern Scandinavia. Conserv. Genet. 9, 581–591.

Jamieson, I.G., Lacy, R.C., 2012. Managing genetic issues in reintroduciton biology. In: Reintroduction Biology: Integrating Science and Management. Wiley-Blackwell, Oxford.
Jombart, T., 2008. Adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24, 1403–1405.
Klopfstein, S., Currat, M., Excoffier, L., 2006. The fate of mutations surfing on the wave of a range expansion. Mol. Biol. Evol. 23, 482–490.
Lennon, N.J., Lintner, R.E., Anderson, S., Alvarez, P., Barry, A., Brockman, W., Daza, R., Erlich, R.L., Giannoukos, G., Green, L., Hollinger, A., Hoover, C.A., Jaffe, D.B., Juhn, F., McCarthy, D., Perrin, D., Ponchner, K., Powers, T.L., Rizzolo, K., Robbins, D., Ryan, E., Russ, C., Sparrow, T., Stalker, J., Steelman, S., Weiand, M., Zimmer, A., Henn, M.R., Nusbaum, C., Nicol, R., 2010. A scalable, fully automated process for construction of sequence-ready barcoded libraries for 454. Genome Biol. 11, R15.
Madsen, T., Ujvari, B., Olsson, M., 2004. Novel genes continue to enhance population growth in adders (*Vipera berus*). Biol. Conserv. 120, 145–147.
Mastretta-Yanes, A., Arrigo, N., Alvarez, N., Jorgensen, T.H., Piñero, D., Emerson, B.C., 2015. Restriction site-associated DNA sequencing, genotyping error estimation and de novo assembly optimization for population genetic inference. Mol. Ecol. Resour. 15, 28–41.
Meirmans, P.G., Van Tienderen, P.H., 2004. GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. Mol. Ecol. Notes 4, 792–794.
Michaelides, S., Cole, N., Funk, S.M., 2015. Translocation retains genetic diversity of a threatened endemic reptile in Mauritius. Conserv. Genet. 16, 661–672.
Mills, L.S., Allendorf, F.W., 1996. The one-migrant-per-generation rule in conservation and management. Conserv. Biol. 10, 1509–1518.
Moraes, A.M., Ruiz-Miranda, C.R., Ribeiro, M.C., Grativol, A.D., Carvalho, C.S., Dietz, J.M., Kierulff, M.C.M., Freitas, L.A., Galetti, P.M., 2017. Temporal genetic dynamics of reintroduced and translocated populations of the endangered golden lion tamarin (*Leontopithecus rosalia*). Conserv. Genet. 1–15.
Moritz, C., Heideman, A., Geffen, E., McRae, P., 1997. Genetic population structure of the Greater Bilby *Macrotis lagotis*, a marsupial in decline. Mol. Ecol. 6, 925–936.
Morton, S.R., 1990. The impact of European settlement on the vertebrate animals of arid Australia: a conceptual model. Proc. Ecol. Soc. Aust. 16, 201–213.
Moseby, K.E., Bice, J.K., 2004. A trial re-introduction of the greater stick-nest rat (*Leporillus conditor*) in arid South Australia. Ecol. Manag. Restor. 5, 118–124.
Moseby, K.E., O'Donnell, E., 2003. Reintroduction of the greater bilby, *Macrotis lagotis* (Reid) (Marsupialia: Thylacomyidae), to northern South Australia: survival, ecology and notes on reintroduction protocols. Wildl. Res. 30, 15–27.
Moseby, K.E., Read, J.L., 2006. The efficacy of feral cat, fox and rabbit exclusion fence designs for threatened species protection. Biol. Conserv. 127, 429–437.
Moseby, K.E., Read, J.L., Paton, D.C., Copley, P., Hill, B.M., Crisp, H.A., 2011. Predation determines the outcome of 10 reintroduction attempts in arid South Australia. Biol. Conserv. 144, 2863–2872.
Newsome, A.E., 1971. Competition between wildlife and domestic livestock. Aust. Vet. J. 47, 577–586.
Pew, J., Muir, P.H., Wang, J., Frasier, T.R., 2015. Related: an R package for analysing pairwise relatedness from codominant molecular markers. Mol. Ecol. Resour. 15, 557–561.
Poland, J.A., Brown, P.J., Sorrells, M.E., Jannink, J.-L., 2012. Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. PLoS One 7, e32253.
Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959.
Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., de Bakker, P.I.W., Daly, M.J., Sham, P.C., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81, 559–575.
Reich, D., Price, A.L., Patterson, N., 2008. Principal component analysis of genetic data. Nat. Genet. 40, 491–492.
Rice, W.R., 1989. Analyzing tables of statistical tests. Evolution 43, 223–225.
Richards, J., 2005. Western Barred Bandicoot, Burrowing Bettong and Banded Hare-Wallaby Recovery Plan 2005–2010. Department of Conservation and Land Management, Wanneroo, Western Australia.
Rivero, E.R., Neves, A.C., Silva-Valenzuela, M.G., Sousa, S.O., Nunes, F.D., 2006. Simple salting-out method for DNA extraction from formalin-fixed, paraffin-embedded tissues. Pathol. Res. Pract. 202, 523–529.
Rohland, N., Reich, D., 2012. Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture. Genome Res. 22, 939–946.
Schwartz, M.K., Luikart, G., Waples, R.S., 2007. Genetic monitoring as a promising tool for conservation and management. Trends Ecol. Evol. 22, 25–33.
Smith, S., Hughes, J., 2008. Microsatellite and mitochondrial DNA variation defines island genetic reservoirs for reintroductions of an endangered Australian marsupial, *Perameles bougainville*. Conserv. Genet. 9, 547–557.
Smith, S., Belov, K., Hughes, J., 2010. MHC screening for marsupial conservation: extremely low levels of class II diversity indicate population vulnerability for an endangered Australian marsupial. Conserv. Genet. 11, 269–278.
Wang, J., 2002. An estimator for pairwise relatedness using molecular markers. Genetics 160, 1203–1215.
Weeks, A.R., Sgro, C.M., Young, A.G., Frankham, R., Mitchell, N.J., Miller, K.A., Byrne, M., Coates, D.J., Eldridge, M.D.B., Sunnucks, P., Breed, M.F., James, E.A., Hoffmann, A.A., 2011. Assessing the benefits and risks of translocations in changing environments: a genetic perspective. Evol. Appl. 4, 709–725.
Weeks, A.R., Moro, D., Thavornkanlapachai, R., Taylor, H.R., White, N.E., Weiser, E.L., Heinze, D., 2015. Conserving and enhancing genetic diversity in translocation programs. In: Advances in Reintroduction Biology of Australian and New Zealand Fauna. CSIRO Publishing, Melbourne.
Weeks, A.R., Stoklosa, J., Hoffmann, A.A., 2016. Conservation of genetic uniqueness of populations may increase extinction likelihood of endangered species: the case of Australian mammals. Front. Zool. 13, 31.
Wright, S., 1931. Evolution in Mendelian populations. Genetics 16, 97–159.